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(21) International Application Number: PCT/US96/09287 (22) International Filing Date: 6 June 1996 (06.06.96) (30) Priority Data: 08/480,120 7 June 1995 (07.06.95) US (71) Applicant: JOHNSON & JOHNSON [US/US]; One Johnson & Johnson Plaza, New Brunswick, NJ 08933-0001 (US). (72) Inventors: JOLIFFE, Linda, K.; 16 Davenport Way, Belle Mead, NJ 08502 (US). ZIVIN, Robert, A.; 6 Glenbrook Court, Lawrenceville, NJ 08648 (US). PULITO, Virginia, L.; 37 Winding Way, Flemington, NJ 08822 (US). (74) Agents: CIAMPORCERO, Audley, A., Jr. et al.; Johnson & Johnson, One Johnson & Johnson Plaza, New Brunswick, NJ 08933-0001 (US).	(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
(54) Title: CDR-GRAFTED ANTI-TISSUE FACTOR ANTIBODIES AND METHODS OF USE THEREOF (57) Abstract The present invention provides CDR-grafted antibodies against human tissue factor that retain the high binding affinity of rodent monoclonal antibodies against tissue factor but have reduced immunogenicity. The present humanized antibodies are potent anticoagulants and are thus useful in the treatment and prophylaxis of human thrombotic disease. The invention also provides methods of making the CDR-grafted antibodies and pharmaceutical compositions for the attenuation or prevention of coagulation.		

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CDR-GRAFTED ANTI-TISSUE FACTOR
ANTIBODIES AND METHODS OF USE THEREOF

FIELD OF THE INVENTION

Monoclonal antibodies capable of inhibiting tissue factor (TF) are useful as anticoagulants. Conventional rodent monoclonal antibodies, however, have limited use in human therapeutic and diagnostic applications due to immunogenicity and short serum half-life. The present invention provides CDR-grafted monoclonal antibodies against TF that retain the high binding affinity of rodent antibodies but have reduced immunogenicity. The present humanized antibodies are potent anticoagulants and are thus useful in the treatment and prophylaxis of human thrombotic disease. The invention also provides methods of making the CDR-grafted antibodies and pharmaceutical compositions for the attenuation or prevention of coagulation.

BACKGROUND OF THE INVENTION

The coagulation of blood involves a cascading series of reactions leading to the formation of fibrin. The coagulation cascade consists of two overlapping pathways, both of which are required for hemostasis. The intrinsic pathway comprises protein factors present in circulating blood, while the extrinsic pathway requires tissue factor, which is expressed on the cell surface of a variety of tissues in response to vascular injury. Davie *et al.*, 1991, Biochemistry 30:10363. Agents that interfere with the coagulation cascade, such

- as heparin and coumarin derivatives, have well-known
1 therapeutic uses in the prophylaxis of venous
thrombosis. Goodman and Gilman, eds., 1980, The
Pharmacological Basis of Therapeutics, MacMillan
Publishing Co., Inc., New York.
- 5 Tissue factor (TF) has been investigated as a
target for anticoagulant therapy. TF is a membrane
glycoprotein that functions as a receptor for factor VII
and VIIa and thereby initiates the extrinsic pathway of
the coagulation cascade in response to vascular injury.
- 10 In addition to its role in the maintenance of hemostasis
by initiation of blood clotting, TF has been implicated
in pathogenic conditions. Specifically, the synthesis
and cell surface expression of TF has been implicated in
vascular disease (Wilcox et al., 1989, Proc. Natl. Acad.
15 Sci. 86:2839) and gram-negative septic shock (Warr et
al., 1990, Blood 75:1481).
- Ruf et al. (1991, Thrombosis and Haemostasis
66:529) characterized the anticoagulant potential of
murine monoclonal antibodies against human TF. The
20 inhibition of TF function by most of the monoclonal
antibodies that were assessed was dependent upon the
dissociation of the TF/VIIa complex that is rapidly
formed when TF contacts plasma. Such antibodies were
thus relatively slow inhibitors of TF in plasma. One
25 monoclonal antibody, TF8-5G9, was capable of inhibiting
the TF/VIIa complex without dissociation of the complex,
thus providing an immediate anticoagulant effect in
plasma. Ruf et al. suggest that mechanisms that
inactivate the TF/VIIa complex, rather than prevent its
30 formation, may provide strategies for interruption of
coagulation in vivo.

The therapeutic use of monoclonal antibodies
1 against TF is limited in that currently available
monoclonals are of rodent origin. The use of rodent
antibodies in human therapy presents numerous problems,
the most significant of which is immunogenicity.
5 Repeated doses of rodent monoclonal antibodies have been
found to elicit an anti-immunoglobulin response termed
human anti-mouse antibody (HAMA), which can result in
immune complex disease and/or neutralization of the
therapeutic antibody. See, e.g., Jaffers et al. (1986)
10 Transplantation 41:572. While the use of human
monoclonal antibodies would address this limitation, it
has proven difficult to generate large amounts of human
monoclonal antibodies by conventional hybridoma
technology.
15 Recombinant technology has been used in an
effort to construct "humanized" antibodies that maintain
the high binding affinity of rodent monoclonal
antibodies but exhibit reduced immunogenicity in humans.
Chimeric antibodies have been produced in which the
20 variable (V) region of a mouse antibody is combined with
the constant (C) region of a human antibody in an effort
to maintain the specificity and affinity of the rodent
antibody but reduce the amount of protein that is non-
human and thus immunogenic. While the immune response
25 to chimeric antibodies is generally reduced relative to
the corresponding rodent antibody, the immune response
cannot be completely eliminated, because the mouse V
region is capable of eliciting an immune response.
Lobuglio et al. (1989) Proc. Natl. Acad. Sci. 86:4220;
30 Jaffers et al. (1986) Transplantation 41:572.

In a recent approach to reducing
1 immunogenicity of rodent antibodies, only the rodent
complementarity determining regions (CDRs), rather than
the entire V domain, are transplanted to a human
antibody. Such humanized antibodies are known as CDR-
5 grafted antibodies. CDRs are regions of
hypervariability in the V regions that are flanked by
relatively conserved regions known as framework (FR)
regions. Each V domain contains three CDRs flanked by
four FRs. The CDRs fold to form the antigen binding
10 site of the antibody, while the FRs support the
structural conformations of the V domains. Thus by
transplanting the rodent CDRs to a human antibody, the
antigen binding domain can theoretically also be
transferred. Owens et al. (1994) J. Immunol. Methods
15 168:149 and Winter et al. (1993) Immunology Today 14:243
review the development of CDR-grafted antibodies.

Orlandi et al. (1989) Proc. Natl. Acad. Sci.
USA 86:3833 constructed a humanized antibody against the
relatively simple hapten nitrophenacetyl (NP). The CDR-
20 grafted antibody contained mouse CDRs and human FRs, and
exhibited NP binding activity similar to the native
mouse antibody. However, the construction of CDR-
grafted antibodies recognizing more complex antigens has
resulted in antibodies having binding activity
25 significantly lower than the native rodent antibodies.
In numerous cases it has been demonstrated that the mere
introduction of rodent CDRs into a human antibody
background is insufficient to maintain full binding
activity, perhaps due to distortion of the CDR
30 conformation by the human FR.

- For example, Gorman et al. (1991) Proc. Natl. Acad. Sci. 88:4181 compared two humanized antibodies against human CD4 and observed considerably different avidities depending upon the particular human framework region of the humanized antibody. Co et al. (1991) Proc. Natl. Acad. Sci. USA 88:2869 required a refined computer model of the murine antibody of interest in order to identify critical amino acids to be considered in the design of a humanized antibody. Kettleborough et al. (1991) Protein Engineering 4:773 report the influence of particular FR residues of a CDR-grafted antibody on antigen binding, and propose that the residues may directly interact with antigen, or may alter the conformation of the CDR loops. Similarly, Singer et al. (1993) J. Immunol. 150:2844 report that optimal humanization of an anti-CD18 murine monoclonal antibody is dependent upon the ability of the selected FR to support the CDR in the appropriate antigen binding conformation. Accordingly, recreation of the antigen-binding site requires consideration of the potential intrachain interactions between the FR and CDR, and manipulation of amino acid residues of the FR that maintain contacts with the loops formed by the CDRs. While general theoretical guidelines have been proposed for the design of humanized antibodies (see, e.g., Owens et al.), in all cases the procedure must be tailored and optimized for the particular rodent antibody of interest.

There is a need in the art for humanized antibodies with reduced immunogenicity and comparable binding affinity relative to the parent rodent antibody for various therapeutic applications. In particular,

there is a need for a humanized antibody against human
1 tissue factor having anticoagulant activity and useful
in the treatment and prevention of thrombotic disease.

SUMMARY OF THE INVENTION

5

The present invention is directed to CDR-
grafted antibodies capable of inhibiting human tissue
factor wherein the CDRs are derived from a non-human
monoclonal antibody against tissue factor and the FR and
10 constant (C) regions are derived from one or more human
antibodies. In a preferred embodiment, the murine
monoclonal antibody is TF8-5G9.

In another embodiment, the present invention
provides a method of producing a CDR-grafted antibody
15 capable of inhibiting human tissue factor which method
comprises constructing one or more expression vectors
containing nucleic acids encoding CDR-grafted antibody
heavy and light chains, transfecting suitable host cells
with the expression vector or vectors, culturing the
20 transfected host cells, and recovering the CDR-grafted
antibody.

The present invention also provides a method
of attenuation of coagulation comprising administering a
CDR-grafted antibody capable of inhibiting human tissue
25 factor to a patient in need of such attenuation.

The present invention further provides a
method of treatment or prevention of thrombotic disease
comprising administering a CDR-grafted antibody capable
of inhibiting human tissue factor to a patient in need
30 of such treatment or prevention. In a preferred

embodiment, the thrombotic disease is intravascular
1 coagulation, arterial restenosis or arteriosclerosis.

Another embodiment of the present invention is
directed to a pharmaceutical composition comprising CDR-
grafted antibodies capable of inhibiting human tissue
5 factor and further comprising a pharmaceutically
acceptable carrier.

BRIEF DESCRIPTION OF THE DRAWINGS

10 Fig. 1 provides the nucleotide and deduced
amino acid sequences of the heavy chain of murine
monoclonal antibody TF8-5G9.

Fig. 2 provides the nucleotide and deduced
amino acid sequences of the light chain of murine
15 monoclonal antibody TF8-5G9.

Fig. 3 is a graph depicting the ability of
CDR-grafted antibody TF8HCDR1 x TF8LCDR1 to bind to
human tissue factor and to compete with murine
monoclonal antibody TF85G9 for binding to tissue factor.
20 Solid symbols indicate direct binding of TF8HCDR1 x
TF8LCDR1 and the positive control chimeric TF85G9 to
tissue factor. Open symbols indicate competition
binding of TF8HCDR1 x TF8LCDR1 or chimeric TF85G9 with
murine monoclonal antibody TF85G9.

25 Fig. 4 presents the DNA sequence of expression
vector pEe6TF8HCDR20 and the amino acid sequence of the
coding regions of the CDR-grafted heavy chain TF8HCDR20.

Fig. 5 presents the DNA sequence of expression
vector pEel2TF8LCDR3 and the amino acid sequence of the
30 coding regions of the CDR-grafted light chain TF8LCDR3.

Fig. 6 is a graph depicting the ability of
1 CDR-grafted antibody TF8HCDR20 x TF8LCDR3 to bind to
human tissue factor.

Fig. 7 is a graph depicting the ability of
CDR-grafted antibody TF8HCDR20 x TF8LCDR3 to compete
5 with murine monoclonal antibody TF85G9 for binding to
tissue factor.

Fig. 8 is a graph depicting the ability of
CDR-grafted antibody TF8HCDR20 x TF8LCDR3 to inhibit
factor X activation.

10 Fig. 9 provides expression vector
pEe6TF8HCDR20 resulting from the subcloning of CDR-
grafted heavy chain TF8HCDR20 into myeloma expression
vector pEehCMV-BglI. The following abbreviations are
used: VH is the CDR-grafted heavy chain variable
15 region; Cy4 is the human IgG4 constant region; pA is the
polyadenylation signal; ampR is the β -lactamase gene;
and hCMV is human cytomegalovirus.

Fig. 10 provides expression vector
pEel2TF8LCDR3 resulting from the subcloning of CDR-
20 grafted light chain TF8LCDR3 into myeloma expression
vector pEel2. The following abbreviations are used: VL
is the CDR-grafted light chain variable region; CK is
the human kappa constant region; SVE is the SV40 early
promoter; GS is glutamine synthetase cDNA. Other
25 abbreviations are as noted in Fig. 9.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides CDR-grafted
30 antibodies capable of inhibiting human tissue factor
wherein the CDRs are derived from a non-human monoclonal

antibody against tissue factor and the FR and C regions
1 are derived from one or more human antibodies. The
present invention further provides methods of making and
using the subject CDR-grafted antibodies.

In accordance with the present invention, the
5 CDR-grafted antibody is an antibody in which the CDRs
are derived from a non-human antibody capable of binding
to and inhibiting the function of human tissue factor,
and the FR and C regions of the antibody are derived
from one or more human antibodies. The CDRs derived
10 from the non-human antibody preferably have from about
90% to about 100% identity with the CDRs of the non-
human antibody, although any and all modifications,
including substitutions, insertions and deletions, are
contemplated so long as the CDR-grafted antibody
15 maintains the ability to bind to and inhibit tissue
factor. The regions of the CDR-grafted antibodies that
are derived from human antibodies need not have 100%
identity with the human antibodies. In a preferred
embodiment, as many of the human amino acid residues as
20 possible are retained in order that immunogenicity is
negligible, but the human residues, in particular
residues of the FR region, are substituted as required
and as taught hereinbelow in accordance with the present
invention. Such modifications as disclosed herein are
25 necessary to support the antigen binding site formed by
the CDRs while simultaneously maximizing the
humanization of the antibody.

Non-human monoclonal antibodies against human
tissue factor from which the CDRs can be derived are
30 known in the art (Ruf et al., 1991; Morrissey et al.,
1988, Thrombosis Research 52:247) or can be produced by

well-known methods of monoclonal antibody production
1 (see, e.g. Harlow et al., eds., 1988, Antibodies, A
Laboratory Manual, Cold Spring Harbor Laboratories, Cold
Spring Harbor, New York). Purified human tissue factor
against which monoclonal antibodies can be raised is
5 similarly well-known (Morrissey et al., 1987, Cell
50:129) and available to the skilled artisan. Murine
monoclonal antibodies, and in particular murine
monoclonal antibody TF8-5G9 disclosed by Ruf et al. and
Morrissey et al., 1988, Thrombosis Research 52:247, and
10 U.S. Patent No. 5,223,427 are particularly preferred.

The ordinarily skilled artisan can determine
the sequences of the CDRs by reference to published
scientific literature or sequence databanks, or by
cloning and sequencing the heavy and light chains of the
15 antibodies by conventional methodology. In accordance
with the present invention, the cDNA and amino acid
sequences of the heavy chain (SEQ ID NOS:1 and 2,
respectively) and light chain (SEQ ID NOS:3 and 4,
respectively) of murine monoclonal antibody TF8-5G9 are
20 provided. The cDNA and deduced amino acid sequence of
the murine TF8-5G9 heavy chain is provided at Figure 1.
The cDNA and deduced amino acid sequence of the murine
TF8-5G9 light chain is provided at Figure 2.

Each of the heavy and light chain variable
25 regions contain three CDRs that combine to form the
antigen binding site. The three CDRs are surrounded by
four FR regions that primarily function to support the
CDRs. The sequences of the CDRs within the sequences of
the variable regions of the heavy and light chains can
30 be identified by computer-assisted alignment according
to Kabat et al. (1987) in Sequences of Proteins of

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Immunological Interest, 4th ed., United States

- 1 Department of Health and Human Services, US Government
 Printing Office, Washington, D.C., or by molecular
 modeling of the variable regions, for example utilizing
 the ENCAD program as described by Levitt (1983) J. Mol.
 5 Biol. 168:595.

In a preferred embodiment the CDRs are derived
 from murine monoclonal antibody TF8-5G9. The preferred
 heavy chain CDRs have the following sequences:

10	CDR1	DDYMH	(SEQ ID NO:5)
	CDR2	LIDPENGNTIYDPKFQG	(SEQ ID NO:6)
	CDR3	DNSYYFDY	(SEQ ID NO:7)

- The preferred light chain CDRs have the following
 15 sequences:

	CDR1	KASQDIRKYLN	(SEQ ID NO:8)
	CDR2	YATSLAD	(SEQ ID NO:9)
	CDR3	LQHGESPYT	(SEQ ID NO:10)

- 20 The sequences of the CDRs of the murine or other non-
 human antibody, and in particular the sequences of the
 CDRs of TF8-5G9, may be modified by insertions,
 substitutions and deletions to the extent that the CDR-
 25 grafted antibody maintains the ability to bind to and
 inhibit human tissue factor. The ordinarily skilled
 artisan can ascertain the maintenance of this activity
 by performing the functional assays described
 hereinbelow. The CDRs can have, for example, from about
 30 50% to about 100% homology to the CDRs of SEQ ID NOS:5-
 10. In a preferred embodiment the CDRs have from about

80% to about 100% homology to the CDRs of SEQ ID NOS:5-10. In a more preferred embodiment the CDRs have from about 90% to about 100% homology to the CDRs of SEQ ID NOS:5-10. In a most preferred embodiment the CDRs have from about 100% homology to the CDRs of SEQ ID NOS:5-10.

The FR and C regions of the CDR-grafted antibodies of the present invention are derived from one or more human antibodies. Human antibodies of the same class and type as the antibody from which the CDRs are derived are preferred. The FR of the variable region of the heavy chain is preferably derived from the human antibody KOL (Schmidt et al., 1983, Hoppe-Seyler's Z. Physiol. Chem. 364:713) The FR of the variable region of the light chain is preferably derived from the human antibody REI (Epp et al., 1974, Eur. J. Biochem. 45:513). In accordance with the present invention, it has been discovered that certain residues of the human FR are preferably replaced by the corresponding residue of the non-human antibody from which the CDRs are derived. For example, certain FR residues of TF8-5G9 are preferably retained to achieve optimal binding to antigen.

For convenience, the numbering scheme of Kabat et al. has been adopted herein. Residues are designated by lower case numbers or hyphens as necessary to conform the present sequences to the standard Kabat numbered sequence.

In accordance with the present invention, residues that are retained in the FR region, i.e. residues that are not replaced by human FR residues, are determined according to the following guidelines. Residues that are idiosyncratic to the parent antibody,

- e.g. TF8-5G9, relative to a human consensus sequence of
- 1 Kabat et al, are retained. Residues of the parent antibody that are in agreement with the consensus sequence are retained if the corresponding residue of the human antibody, e.g. KOL or REI, is idiosyncratic.
 - 5 Residues that are part of the antibody loop canonical structures defined by Chothia et al. (1989) Nature 342:877, such as residue 71 of the heavy and light chains, are retained. FR residues predicted to form loops, such as residues 28-30 of the heavy chain, are
 - 10 retained. FR residues predicted to influence the conformation of the CDRs such as residues 48 and 49 preceding CDR2 of the heavy chain, are retained. Residues that have been demonstrated to be critical in the humanization of other antibodies may also be
 - 15 retained. The foregoing guidelines are followed to the extent necessary to support the antigen binding site formed by the CDRs while simultaneously maximizing the humanization of the antibody.

The amino acid sequence of a representative

- 20 CDR-grafted heavy chain variable region derived from murine monoclonal antibody TF8-5G9 and human antibody KOL is shown below. The CDR-grafted heavy chain is designated TF8HCDR1; murine residues were retained in the FR at residues 6, 17, 23, 24, 28, 29, 30, 48, 49,
- 25 68, 71, 73, 78, 88 and 91. CDRs are underlined.

	10	20	30	35ab	50
	QVQLVQSGGG	VVQPGRLRL	SCKASGFNIK	<u>DYYMH</u> --WVR	QAPGKGLEWIG
	52abc	60	70	80 82abc	90
	<u>LIDP</u> --ENGNTIYD	<u>PKFQGRFSIS</u>	ADTSK--NTAFL	QMDSLRPEDTAVY	
	100	110			
30	YCARDNSYYF	<u>DYWGQ</u> GPVT	VSS	(SEQ ID NO:11)	

The amino acid sequence of a representative
 1 CDR-grafted light chain variable region derived from
 murine monoclonal antibody TF8-5G9 and human antibody
 REI is shown below. The CDR-grafted light chain is
 designated TF8LCDR1; murine residues were retained in
 5 the FR at residues 39, 41, 46 and 105. CDRs are
 underlined.

	10	20	30	40	50
	DIQMTQSPSS	LSASVGDRVT	<u>ITCKASQDIR</u>	<u>KYLNWYQOK</u>	<u>WKAPKTLIYY</u>
10	60	70	80	90	100
	<u>ATSLADGVPS</u>	RFSGSGSGTD	YTFTISSLPQ	<u>EDIATYYCLO</u>	<u>HGESPYTFGQ</u>
	GTKLEITR (SEQ ID NO:12)				

A CDR-grafted antibody containing variable
 15 regions TF8HCDR1 and TF8LCDR1 has been demonstrated in
 accordance with the present invention to be as effective
 as murine monoclonal antibody TF8-5G9 in binding to
 human tissue factor. It has been further discovered in
 accordance with the present invention, by examination of
 20 the molecular structure of murine monoclonal antibody
 TF8-5G9, and by design, construction, and analysis of
 CDR-grafted antibodies, that the FR regions can be
 further humanized without the loss of antigen binding
 activity. In particular, the FR region may retain the
 25 human FR residue at residues 6, 17, 68, 73 and 78 of the
 heavy chain, and residues 39, 41, 16 and 105 of the
 light chain, with maintenance of antigen binding
 activity.

In a most preferred embodiment, the heavy
 30 chain variable region contains a FR derived from human
 antibody KOL in which murine monoclonal antibody TF8-5G9

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residues are retained at amino acids 23, 24, 28, 29, 30,
 1 48, 49, 71, 88 and 91. The preferred heavy chain
 variable region is designated TF8HCDR20 and has the
 following sequence.

```

5          10          20          30    35ab          50
  QVQLVESGGG VVQGRSLRL SCKASGFNIK DYYMH--WVR QAPGKGLEWIGL

52abc      60          70          80 82abc      90          100
  IDP--ENGNTIYD PKFQGRFTIS ADNSKNTLFL QMDSLRPEDTAVY YCARDNSIYF

10          110
  DYWGQGTPVT VSS (SEQ ID NO:13)
  
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In a most preferred embodiment, the light
 chain variable region contains a FR derived from human
 15 antibody REI in which murine monoclonal antibody TF8-5G9
 residues are retained at amino acids 39 and 105. The
 preferred light chain variable region is designated
 TF8LCDR20 and has the following sequence.

```

          10          20          30          40          50
20  DIQMTQSPSS LSASVGDRV ITCKASQDIR KYLNWYQQKP GKAPKLLIYY
          60          70          80          90          100
  ATSLADGVPS RFSGSGSGTD YTFTISSLQP EDIATYYCLQ HGESPYTFGQ
  GTKLEITR (SEQ ID NO:14)
  
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It is within the ken of the ordinarily skilled
 25 artisan to make minor modifications of the foregoing
 sequences, including amino acid substitutions, deletions
 and insertions. Any such modifications are within the
 scope of the present invention so long as the resulting
 CDR-grafted antibody maintains the ability to bind to
 30 and inhibit human tissue factor. The ordinarily skilled
 artisan can assess the activity of the CDR-grafted

antibody with reference to the functional assays
1 described hereinbelow.

The human constant region of the CDR-grafted
antibodies of the present invention is selected to
minimize effector function. The intended use of the
5 CDR-grafted antibodies of the present invention is to
block the coagulation cascade by inhibition of tissue
factor, and thus antibody effector functions such as
fixation of complement are not desirable. Antibodies
with minimal effector functions include IgG2, IgG4, IgA,
10 IgD and IgE. In a preferred embodiment of the present
invention, the heavy chain constant region is the human
IgG4 constant region, and the light chain constant
region is the human IgG4 kappa constant region.

In that effector functions may not be
15 desirable for therapeutic uses, the present invention
further contemplates active fragments of the CDR-grafted
antibodies, and in particular Fab fragments and F(ab')₂
fragments. Active fragments are those fragments capable
of inhibiting human tissue factor. Fab fragments and
20 F(ab')₂ fragments may be obtained by conventional means,
for example by cleavage of the CDR-grafted antibodies of
the invention with an appropriate proteolytic enzyme
such as papain or pepsin, or by recombinant production.
The active fragments maintain the antigen binding sites
25 of the CDR-grafted antibodies and thus are similarly
useful therapeutically.

The ability of the CDR-grafted antibodies
designed and constructed as taught in accordance with
the present invention to bind and inhibit human tissue
30 factor can be assessed by functional assays. For
example, in a rapid and convenient assay, expression

vectors containing nucleic acids encoding the CDR-
1 grafted heavy and light chains can be co-transfected
into suitable host cells and transiently expressed. The
resulting antibodies can be assessed by standard assays
for ability to bind human tissue factor, and for ability
5 to compete for binding to tissue factor with the non-
human antibody from which the CDRs are derived.

For example, transient expression of nucleic
acids encoding the CDR-grafted heavy and light chains in
COS cells provides a rapid and convenient system to test
10 antibody gene expression and function. Nucleic acids
encoding the CDR-grafted heavy and light chains,
respectively, are cloned into a mammalian cell
expression vector, for example pSG5, described by Green
et al. (1988) Nucleic Acids Res. 16:369 and commercially
15 available from Stratagene Cloning Systems, La Jolla, CA.
The pSG5 expression vector provides unique restriction
sites for the insertion of the heavy and light chain
genes, and in vivo expression is under the control of
the SV40 early promoter. Transcriptional termination is
20 signaled by the SV40 polyadenylation signal sequence.

The pSG5-based expression vectors containing
nucleic acids encoding the heavy and light chains are
cotransfected into COS cells and cultured under
conditions suitable for transient expression. Cell
25 culture media is then harvested and examined for
antibody expression, for example by an enzyme linked
immunosorbent assay (ELISA), to determine that suitable
levels of antibody have been produced. An ELISA may
then be used to assess the ability of the CDR-grafted
30 antibody to bind to human tissue factor. Human tissue
factor is immobilized on a microtiter plate and the COS

cell supernatant containing the CDR-grafted antibody is
1 added followed by an incubation at room temperature for
about one hour. The plates are then washed with a
suitable detergent-containing buffer such as phosphate
buffered saline (PBS)/Tween, followed by the addition of
5 the components of a suitable detection system. For
example, horseradish peroxidase conjugated goat anti-
human kappa chain polyclonal antibody is added, followed
by washing, followed by addition of substrate for
horseradish peroxidase, and detection. The CDR-grafted
10 antibodies within the scope of the present invention are
those which are capable of binding to human tissue
factor to a degree comparable to the non-human antibody
from which the CDRs are derived as determined by the
foregoing assay.

15 The ability of the CDR-grafted antibodies to
inhibit the activity of human tissue factor in vivo can
be conveniently assessed by the following in vitro assay
that mimics in vivo coagulation events. In response to
vascular injury in vivo, tissue factor binds to factor
20 VII and facilitates the conversion of factor VII to a
serine protease (factor VIIa). The factor VIIa-tissue
factor complex converts factor X to a serine protease
(factor Xa). Factor Xa forms a complex with factor Va
(from the intrinsic coagulation pathway), resulting in
25 the conversion of prothrombin to thrombin, which in turn
results in the conversion of fibrinogen to fibrin. In a
convenient in vitro functional assay, tissue factor is
incubated in the presence of factor VIIa and the CDR-
grafted anti-tissue factor antibody produced in the
30 transient expression system described above. Factor X
is added and the reaction mixture is incubated, followed

by an assay for factor Xa activity utilizing a
1 chromogenic substrate for factor Xa (Spectrozyme FXa,
American Diagnostica, Inc., Greenwich, CT). The ability
of the CDR-grafted antibody to inhibit factor X
activation thus provides a measure of the ability of the
5 CDR-grafted antibody to inhibit the activity of human
tissue factor.

The CDR-grafted antibodies within the scope of
the present invention are those which are capable of
inhibiting human tissue factor to a degree comparable to
10 the non-human antibody from which the CDRs are derived
as determined by the foregoing assay. In one
embodiment, the CDR-grafted antibody has at least 50% of
the inhibitory activity of TF8-5G9 for human tissue
factor. In a preferred embodiment, the CDR-grafted
15 antibody has at least 70% of the inhibitory activity of
TF8-5G9 for human tissue factor. In a more preferred
embodiment, the CDR-grafted antibody has at least 80% of
the inhibitory activity of TF8-5G9 for human tissue
factor. In a most preferred embodiment, the CDR-grafted
20 antibody has at least 90% of the inhibitory activity of
TF8-5G9 for human tissue factor.

In another embodiment, the present invention
provides a method of producing a CDR-grafted antibody
capable of inhibiting human tissue factor. The method
25 comprises constructing an expression vector containing a
nucleic acid encoding the CDR-grafted antibody heavy
chain and an expression vector containing a nucleic acid
encoding the CDR-grafted antibody light chain,
transfecting suitable host cells with the expression
30 vectors, culturing the transfected host cells under
conditions suitable for the expression of the heavy and

light chains, and recovering the CDR-grafted antibody.

- 1 Alternately, one expression vector containing nucleic acids encoding the heavy and light chains may be utilized.

- Standard molecular biological techniques, for
5 example as disclosed by Sambrook et al. (1989),
Molecular Cloning: A Laboratory Manual Cold Spring Harbor Press, Cold Spring Harbor, NY may be used to obtain nucleic acids encoding the heavy and light chains of the CDR-grafted antibodies of the present invention.
10 A nucleic acid encoding the CDR-grafted variable domain may be constructed by isolating cDNA encoding the antibody to be humanized, e.g. murine monoclonal antibody TF8-5G9, by conventional cloning methodology from the hybridoma producing the antibody, or by
15 polymerase chain reaction (PCR) amplification of the variable region genes, as described for example by Winter et al., followed by site-directed mutagenesis to substitute nucleotides encoding the desired human residues into the FR regions. Alternately, the cDNA
20 encoding the human antibody can be isolated, followed by site-directed mutagenesis to substitute nucleotides encoding the desired murine residues into the CDRs.

- Nucleic acids encoding the CDR-grafted variable domain may also be synthesized by assembling
25 synthetic oligonucleotides, for example utilizing DNA polymerase and DNA ligase. The resulting synthetic variable regions may then be amplified by PCR. Nucleic acids encoding CDR-grafted variable domains may also be constructed by PCR strand overlap methods that are known
30 in the art and reviewed by Owens et al.

Accordingly, having determined the desired
1 amino acid sequences of the CDR-grafted variable domains
in accordance with the present invention, the ordinarily
skilled artisan can obtain nucleic acids encoding the
variable domains. Further, the skilled artisan is aware
5 that due to the degeneracy of the genetic code, various
nucleic acid sequences can be constructed that encode
the CDR-grafted variable domains. All such nucleic acid
sequence are contemplated by the present invention.

The nucleic acids encoding the CDR-grafted
10 variable domains are linked to appropriate nucleic acids
encoding the human antibody heavy or light chain
constant region. Nucleic acid sequences encoding human
heavy and light chain constant regions are known in the
art. It is within the ken of the ordinarily skilled
15 artisan to include sequences that facilitate
transcription, translation and secretion, for example
start codons, leader sequences, the Kozak consensus
sequence (Kozak, 1987, J. Mol. Biol. 196:947) and the
like, as well as restriction endonuclease sites to
20 facilitate cloning into expression vectors.

The present invention thus further provides
nucleic acids encoding the heavy and light chains of
CDR-grafted antibodies capable of inhibiting human
tissue factor wherein the CDRs are derived from a murine
25 monoclonal antibody against tissue factor and the FR and
C regions are derived from one or more human antibodies.

In accordance with the present invention,
representative nucleic acids encoding CDR-grafted heavy
and light chains were constructed. The CDR-grafted
30 heavy chain comprises a variable region containing FR
regions derived from human antibody KOL and CDRs derived

from murine monoclonal antibody TF8-5G9 and further
1 comprises a constant region derived from the heavy chain
of human IgG4. The CDR-grafted light chain comprises a
variable region containing FR regions derived from human
antibody REI and CDRs derived from murine monoclonal
5 antibody TF8-5G9 and further comprises a constant region
derived from human IgG4 kappa chain. Nucleic acids
encoding the heavy and light chains were constructed by
assembling the variable regions from synthetic
nucleotides, amplifying the assembled variable regions
10 by PCR, purifying the amplified nucleic acids, and
ligating the nucleic acid encoding the variable region
into a vector containing a nucleic acid encoding the
appropriate human constant region.

The sequences of representative nucleic acids
15 encoding CDR-grafted heavy and light chains are
presented as nucleotides 1-2360 of SEQ ID NO:15 and
nucleotides 1-759 of SEQ ID NO:20, respectively.

The nucleic acid sequence encoding a preferred
heavy chain (nucleotides 1-2360 of SEQ ID NO:15) is
20 designated the TF8HCDR20 gene. The nucleic acid
sequence contains the following regions: 5' EcoRI
restriction site (nucleotides 1-6); Kozak sequence
(nucleotides 7-15); start codon and leader sequence
(nucleotides 16-72); CDR-grafted variable region
25 (nucleotides 73-423); human IgG4 CH1 domain (nucleotides
424-717); human IgG4 intron 2 (nucleotides 718-1110);
human IgG4 hinge (nucleotides 1111-1146); human IgG4
intron 3 (nucleotides 1147-1267); human IgG4 CH2 domain
(nucleotides 1268-1594); human IgG4 intron 4
30 (nucleotides 1595-1691); human IgG4 CH3 domain
(nucleotides 1692-2012); 3' untranslated region

(nucleotides 2013-2354); 3' BamHI end spliced to BclI
1 site of expression vector (nucleotides 2355-2360).

The nucleic acid sequence encoding a preferred
light chain gene (nucleotides 1-759 of SEQ ID NO:20) is
designated the TF8LCDR3 gene. The nucleic acid sequence
5 contains the following regions: 5' EcoRI restriction
site (nucleotides 1-5); Kozak sequence (nucleotides 6-
8); start codon and leader sequence (nucleotides 9-68);
CDR-grafted variable region (nucleotides 69-392); human
kappa constant region (nucleotides 393-710); 3'
10 untranslated region (nucleotides 711-753); 3' BamHI end
spliced to BclI site of expression vector (nucleotides
754-759).

The foregoing preferred sequences can be
modified by the ordinarily skilled artisan to take into
15 account degeneracy of the genetic code, and to make
additions, deletions, and conservative and
nonconservative substitutions that result in a
maintenance of the function of the nucleic acid, i.e.
that it encodes a heavy or light chain of a CDR-grafted
20 antibody capable of inhibiting human tissue factor.
Restriction sites and sequences that facilitate
transcription and translation may be altered or
substituted as necessary depending upon the vector and
host system chosen for expression.

25 Suitable expression vectors and hosts for
production of the CDR-grafted antibodies of the present
invention are known to the ordinarily skilled artisan.
The expression vectors contain regulatory sequences,
such as replicons and promoters, capable of directing
30 replication and expression of heterologous nucleic acids
sequences in a particular host cell. The vectors may

also contain selection genes, enhancers, signal
1 sequences, ribosome binding sites, RNA splice sites,
polyadenylation sites, transcriptional terminator
sequences, and so on. The vectors may be constructed by
conventional methods well-known in the art, or obtained
5 from commercial sources. The expression vectors
preferably have convenient restriction sites at which
the nucleic acids encoding the antibody chains of the
invention are inserted. Myeloma expression vectors in
which antibody gene expression is driven by the human
10 cytomegalovirus promoter-enhancer or are particularly
preferred.

Expression vectors containing a nucleic acid
encoding the CDR-grafted heavy chain under the control
of a suitable promoter and expression vectors containing
15 a nucleic acid encoding the CDR-grafted light chain
under the control of a suitable promoter are
cotransfected into a suitable host cell. In another
embodiment, nucleic acids encoding both heavy and light
chains are provided in a single vector for transfection
20 of a suitable host cell.

Suitable host cells or cell lines for
expression of the CDR-grafted antibodies of the present
invention include bacterial cells, yeast cells, insect
cells, and mammalian cells such as Chinese hamster ovary
25 (CHO) cells, COS cells, fibroblast cells and myeloid
cells. Mammalian cells are preferred. CHO, COS and
myeloma cells are particularly preferred. Myeloma cells
are preferred for establishing permanent CDR-grafted
antibody producing cell lines. Expression of antibodies
30 in myeloma cells, bacteria, and yeast is reviewed by

Sandhu (1992) Critical Reviews in Biotechnology 12:437.

1 Expression in mammalian cells is reviewed by Owen et al..

Transfection of host cells by the expression
vectors containing nucleic acids encoding the CDR-
grafted heavy and light chains can be accomplished by
5 methods well-known to one of ordinary skill in the art.
Such methods include, for example, calcium chloride
transfection, calcium phosphate transfection,
lipofection and electroporation. Suitable culture
methods and conditions for the production of the CDR-
10 grafted antibodies are likewise well-known in the art.
The CDR-grafted antibodies can be purified by
conventional methods, including ammonium sulfate
precipitation, affinity chromatography, gel
electrophoresis, and the like. The ability of the CDR-
15 grafted antibodies to bind to and inhibit human tissue
factor can be assessed by the in vitro assays described
above.

The CDR-grafted antibodies of the present
invention have a variety of utilities. For example, the
20 antibodies are capable of binding to human tissue factor
and thus are useful in assays for human tissue factor
from body fluid samples, purification of human tissue
factor, and so on.

The CDR-grafted antibodies of the present
25 invention are capable of inhibiting human tissue factor.
Human tissue factor is well-known to be an essential
element in the human coagulation cascade. The ability
of the antibodies of the present invention to disrupt
the coagulation cascade is demonstrated by in vitro
30 assays in which the antibodies prevent factor X
activation. Accordingly, the present antibodies are

useful in the attenuation of coagulation. The present
1 invention thus provides a method of attenuation of
coagulation comprising administering a therapeutically
effective amount of CDR-grafted antibody capable of
inhibiting human tissue factor to a patient in need of
5 such attenuation.

Numerous thrombotic disorders are
characterized by excessive or inappropriate coagulation
and are effectively treated or prevented by
administration of agents that interfere with the
10 coagulation cascade. Accordingly, the present invention
further provides a method of treatment or prevention of
a thrombotic disorder comprising administering a
therapeutically effective amount of a CDR-grafted
antibody capable of inhibiting human tissue factor to a
15 patient in need of such treatment or prevention. In a
preferred embodiment, the thrombotic disorder is
intravascular coagulation, arterial restenosis or
arteriosclerosis. The antibodies of the invention may be
used in combination with other antibodies or therapeutic
20 agents.

A therapeutically effective amount of the
antibodies of the present invention can be determined by
the ordinarily skilled artisan with regard to the
patient's condition, the condition being treated, the
25 method of administration, and so on. A therapeutically
effective amount is the dosage necessary to alleviate,
eliminate, or prevent the thrombotic disorder as
assessed by conventional parameters. For example, a
therapeutically effective dose of a CDR-grafted antibody
30 of the present invention may be from about 0.1 mg to
about 20 mg per 70 kg of body weight. A preferred

dosage is about 1.0 mg to about 5 mg per 70 kg of body
1 weight.

A patient in need of such treatment is a
patient suffering from a disorder characterized by
inappropriate or excessive coagulation, or a patient at
5 risk of such a disorder. For example, anticoagulant
therapy is useful to prevent postoperative venous
thrombosis, and arterial restenosis following balloon
angioplasty.

The CDR-grafted antibodies of the present
10 invention are useful in the same manner as comparable
therapeutic agents, and the dosage level is of the same
order of magnitude as is generally employed with those
comparable therapeutic agents. The present antibodies
may be administered in combination with a
15 pharmaceutically acceptable carrier by methods known to
one of ordinary skill in the art.

Another embodiment of the present invention is
directed to a pharmaceutical composition comprising a
least one CDR-grafted antibody capable of inhibiting
20 human tissue factor and further comprising a
pharmaceutically acceptable carrier. As used herein,
"pharmaceutically acceptable carrier" includes any and
all solvents, dispersion media, coatings, antibacterial
and antifungal agents, isotonic and absorption delaying
25 agents, and the like. The use of such media and agents
for pharmaceutically active substances is well-known in
the art. Except insofar as any conventional media or
agent is incompatible with the active ingredient, its
use in the therapeutic compositions is contemplated.
30 Supplementary active ingredients can also be
incorporated into the compositions.

The antibodies can be administered by well-
1 known routes including oral and parenteral, e.g.,
intravenous, intramuscular, intranasal, intradermal,
subcutaneous, and the like. Parenteral administration
and particularly intravenous administration is
5 preferred. Depending on the route of administration,
the pharmaceutical composition may require protective
coatings.

The pharmaceutical forms suitable for
injectionable use include sterile aqueous solutions or
10 dispersions and sterile powders for the extemporaneous
preparation of sterile injectable solutions or
dispersions. In all cases the ultimate solution form
must be sterile and fluid. Typical carriers include a
solvent or dispersion medium containing, for example,
15 water buffered aqueous solutions (i.e., biocompatible
buffers), ethanol, polyol such as glycerol, propylene
glycol, polyethylene glycol, suitable mixtures thereof,
surfactants or vegetable oils. The antibodies may be
incorporated into liposomes for parenteral
20 administration. Sterilization can be accomplished by an
art-recognized techniques, including but not limited to,
addition of antibacterial or antifungal agents, for
example, paraben, chlorobutanol, phenol, sorbic acid or
thimersal. Further, isotonic agents such as sugars or
25 sodium chloride may be incorporated in the subject
compositions.

Production of sterile injectable solutions
containing the subject antibodies is accomplished by
incorporating these antibodies in the required amount in
30 the appropriate solvent with various ingredients
enumerated above, as required, followed by

sterilization, preferably filter sterilization. To
1 obtain a sterile powder, the above solutions are vacuum-
dried or freeze-dried as necessary.

The following examples further illustrate the
present invention.

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EXAMPLE 1

1 Isolation and Sequencing of TF8-5G9
 Light Chain (LC) and Heavy Chain (HC)

 Two DNA libraries were generated from oligo
5 (dT)-primed TF8-5G9 hybridoma RNA utilizing standard
molecular biology procedures as described by Sambrook et
al. The cDNA was cloned into the Librarian II plasmid
vector from Invitrogen (San Diego, CA), and the
libraries were screened for cDNA clones encoding murine
10 IgG HC and LC. A full-length cDNA clone for the heavy
chain could not be isolated, despite the construction of
two independent libraries. A random primed TF8-5G9 cDNA
library was generated to obtain the missing 5' sequence
of the heavy chain. Consequently, the heavy chain cDNA
15 was in two pieces: a 5' clone of 390 nucleotides and a
3' clone of 1392 nucleotides. The two HC clones overlap
by 292 nucleotides.

 The HC and LC clones were completely sequenced
by the dideoxy chain termination method of Sanger et al.
20 (1977) Proc. Natl. Acad. Sci. USA 74:5463. To verify
the variable region sequence, sequence was obtained from
PCR-amplified cDNA that had been synthesized from total
TF8-5G9 hybridoma RNA. Total TF8-5G9 hybridoma RNA was
isolated by the guanidinium thiocyanate method of
25 Chrigwin et al. (1970) Biochemistry 18:5294. cDNA was
synthesized using the Perkin Elmer (Norwalk, CT) GeneAmp
RNA Polymerase Chain Reaction (PCR) kit with an oligo
(dT) primer. Components of the same kit were used in
the PCR to amplify the LC and HC variable regions using
30 primers based on the sequence that had been obtained for
the cDNA clones. The amplified variable region

fragments were gel-purified and sequenced according to
1 the method of Tracy et al. (1991) BioTechniques 11:68 on
a Model 373A Applied Biosystems, Inc. (Foster City, CA)
automated fluorescent DNA sequencer. The sequence for
TF8-5G9 LC and HC obtained from RNA amplification and
5 the sequence obtained from the cDNA clones agreed. The
TF8-5G9 HC variable region sequence with protein
translation is shown in Figure 1 and SEQ ID NO:1, and
that for the LC is shown in Figure 2 and SEQ ID NO:3.

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EXAMPLE 2

1 Chimeric LC and HC Expression Vector Construction

 In order to test the binding activity of the CDR-grafted anti-TF LC and HC individually, mouse-human
5 chimeric TF8-5G9 LC and HC were constructed. This allowed the CDR-grafted LC to be tested for TF binding ability in combination with the chimeric HC, and the CDR-grafted HC to be tested in combination with the chimeric LC.

10 Primers were designed to amplify the TF8-5G9 LC variable region using as template cDNA clones in the Librarian II vector. The 5' primer was designed with an EcoRI site while the 3' primer was designed with a NarI site. PCR was used to amplify the LC variable region,
15 generating a 433 bp fragment with a 5'EcoRI end and 3'NarI end. The fragment included the signal sequence from the TF8-5G9 LC cDNA clone but incorporated a 2 base change in the arginine codon immediately following the ATG start codon. This change retained the arginine
20 residue but made the sequence conform to the Kozak consensus sequence in order to potentially improve translation of the LC mRNA. The PCR amplified LC variable region fragment was digested with EcoRI and NarI restriction enzymes and purified by electrophoresis
25 on a 2% Nusieve, 1% Seakem agarose gel (FMC Bio Products, Rockland, ME).

 The DNA was extracted from the gel slice and purified by the Geneclean (Bio 101, La Jolla, CA) procedure. The full-length chimeric TF8-5G9 LC gene was
30 generated by cloning this DNA into the EcoRI and NarI sites of a pSP73 vector (Promega, Madison, WI) which

contains the human kappa constant region. The gene was
1 isolated from the pSP73 vector by EcoRI digestion and
subcloned into the EcoRI site of the pSG5 mammalian cell
expression vector (Stratagene Cloning Systems, La Jolla,
CA).

5 The chimeric TF8-5G9 HC gene was assembled in
a manner similar to that of the chimeric LC. Since
there was no full-length HC cDNA isolated from the
Librarian II vector cDNA libraries, the HC variable
region fragment that was generated by the PCR from total
10 TF8-5G9 hybridoma cell RNA was used as the template.
Primers which incorporated an EcoRI site at the 5' end
and a SacI site at the 3' end were used in the PCR to
generate a 430 bp fragment which contained the TF8-5G9
HC Kozak sequence, start codon, signal sequence, and
15 variable region. This fragment was digested with the
restriction enzymes EcoRI and SacI, and gel-purified
using the same procedure that was used with the chimeric
LC construction.

The full-length TF8-5G9 chimeric HC gene was
20 constructed by cloning the variable region fragment into
the EcoRI and SacI sites of the pSG5 expression vector
containing the human IgG4 constant region.

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EXAMPLE 3

1 Design and Construction of the
 CDR-Grafted Heavy and Light Chain Genes

 The variable region domains of the CDR-grafted
5 HC and LC genes were designed with an EcoRI overhang at
the 5' end followed by a Kozak sequence to improve
antibody expression. The leader sequences were derived
from the heavy and light chains of the murine monoclonal
antibody B72.3 (Whittle et al. (1987) Protein
10 Engineering 1:499). The 3' end of the variable regions
were designed to have overhangs which allowed for
splicing to the appropriate human constant region DNA.

 In the initially designed CDR-grafted TF8-5G9
heavy and light chains the CDRs were derived from murine
15 TF8-5G9 sequence while the frameworks were derived
primarily from human antibody sequence. The human
antibody KOL (Schmidt et al.) was used for the heavy
chain frameworks, while the human antibody dimer (Epp et
al.) was used for the light chain frameworks.

20 Several criteria were used to select murine
framework residues in the design of the TF8-5G9 CDR-
grafted heavy and light chain variable regions.
Framework residues which, at a particular position, are
idiosyncratic to TF8-5G9 were retained as murine
25 sequence with the assumption that they contributed to
its unique binding characteristics. TF8-5G9 murine
residues were also retained at framework positions where
they were in agreement with the human consensus sequence
but where the corresponding residues in KOL or REI were
30 idiosyncratic. Residues that are part of antibody loop
canonical structures such as residue 71 (numbering

according to Kabat et al.) of the heavy and light chains
1 were also retained as murine sequence. Framework
residues that form loops such as residues 26-30 of the
HC were kept as TF8-5G9 murine sequence at positions
where the murine sequence differed from the human.
5 Residues known to directly influence the conformation of
CDRs, such as 48 and 49 immediately preceding CDR2 of
the HC, were also retained as murine sequence.

The amino acid sequence of the variable region
for the initially designed CDR-grafted TF8-5G9 HC,
10 TF8HCDR1, is shown in SEQ ID NO:11. Murine residues
were retained at framework positions 6, 17, 23, 24, 28,
29, 30, 48, 49, 68, 71, 73, 78 88 and 91. The CDR-
grafted HC variable region was attached to a human IgG4
constant region.

15 The amino acid sequence of the variable region
for the initially designed CDR-grafted TF8-5G9 LC,
TF8LCDR1, is shown in SEQ ID NO:12. Murine residues
were retained at framework positions 39, 41, 46 and 105.
The CDR-grafted LC variable region was attached to a
20 human kappa constant region.

The variable region for the CDR-grafted HC and
LC described above were each assembled from 13 synthetic
oligonucleotides which were synthesized by Research
Genetics, Inc., Huntsville, AL. These oligonucleotides
25 ranged in length from 42 to 80 bases, and encoded both
variable region strands. When the 6 complementary
oligonucleotide pairs were annealed, the overhangs
generated were 17 to 24 bases in length. These
oligonucleotide pairs were combined, annealed at their
30 complementary overhangs, and ligated to give the final
full length double-stranded variable regions.

The HC variable region oligonucleotides were
1 assembled into a 452 bp fragment which contains a 5'
EcoRI site and a 3' SacI site. The polymerase chain
reaction was used to amplify this fragment. The
resulting amplified DNA was purified on a 2% Nusieve, 1%
5 Seakem agarose gel (FMC). The appropriate size band of
DNA was excised and the DNA was recovered by the
Geneclean (Bio 101) procedure. The fragment was then
digested with EcoRI and SacI, and purified again by the
Geneclean method. This HC variable region fragment with
10 EcoRI and SacI ends was cloned into the EcoRI and SacI
sites of the pSport-1 vector (GIBCO-BRL Life
Technologies, Gaithersburg, MD). DNA from several
clones was isolated and sequenced to verify proper
variable region assembly. All clones had unexpected
15 base changes. One clone with the fewest base changes
(two mismatches at bases 133 and 140) was selected to be
corrected by site-directed mutagenesis according to
Kunkel (1985) Proc. Natl. Acad. Sci. USA 82:488.
Briefly, CJ236 (ung-, dut-) competent cells (Invitrogen
20 Corporation, San Diego, CA) were transformed with the
pSport vector containing the CDR-grafted HC variable
region with the two base mismatch. Single-stranded,
uridine-incorporated DNA templates were purified from
phage following M13 helper phage (Stratagene Cloning
25 Systems) infection of the transformed cells.
Mutagenesis oligos containing the desired base changes
were synthesized on an Applied Biosystems Model 380B DNA
synthesizer. The mutagenesis oligos were annealed to
the template DNA, and T7 DNA Polymerase and T4 DNA
30 Ligase (MutaGene InVitro Mutagenesis Kit, Bo-Rad
Laboratories, Richmond, CA) were used to incorporate the

oligo into a newly synthesized DNA strand. DH5 α
1 competent cells (GIBCO-BRL Life Technologies) were
transformed with the double-stranded DNA. The original
uridine-incorporated strand is destroyed while the newly
synthesized strand containing the mutagenesis oligo is
5 replicated. Phagemid DNA was prepared from the
resulting mutagenesis clones and the variable regions
were sequence to identify the clones which had
incorporated the desired changes. The corrected HC
EcoRI/SacI variable region fragment was excised from the
10 pSport vector, purified and ligated into the EcoRI/SacI
sites of a pSG5 vector containing the human IgG4
constant region. This resulted in the generation of a
full-length humanized TF8-5G9 HC gene, TF8HCDR1, in the
pSG5 COS cell expression vector. The vector was
15 designated pSG5TF8HCDR1.

The CDR-grafted TF8-5G9 LC variable region was
also amplified by the PCR from the assembled synthetic
oligonucleotides into a 433 bp fragment which contained
a 5' EcoRI site and a 3' NarI site. This fragment was
20 purified as described above for the HC, digested with
EcoRI and NarI and purified by the Geneclean procedure.
This fragment was cloned into the EcoRI and NarI sites
of a pSG5 vector which contains the human kappa constant
region. This resulted in the generation of a full-
25 length humanized TF8-5G9 LC gene, TF8LCDR1, in the pSG5
COS cell expression vector. Seven clones were
sequenced, and one was found to have the desired CDR-
grafted LC sequence. The vector was designated
pSQ5TF8LCDR1.

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EXAMPLE 4

1 **Expression of the CDR-Grafted
Heavy and Light Chain Genes in COS Cells**

5 The transient expression of antibody genes in
COS-1 cells provides a rapid and convenient system to
test antibody gene expression and function. COS-1 cells
were obtained from the American Type Culture Collection
(CRL 1650) and cultured in Dulbecco's Modified Eagle
Medium (DMEM, from GIBCO BRL Life Technologies) with 10%
10 fetal calf serum. The pSG5TF8HCDR1 expression factor
was cotransfected into COS cells with the pSG5 chimeric
LC expression vector using the DEAE-Dextran method
followed by DMSO shock as described by Lopata et al.
(1984) Nucleic Acids Res. 14:5707. After 4 days of
15 culture, media was harvested from the wells and examined
for antibody expression levels.

Antibody levels were determined by an ELISA-
based assembly assay. Plates were coated with a goat
anti-human Fc specific antibody. Various dilutions of
20 the COS cell supernatant containing secreted antibody
were added, incubated for one hour, and washed. A
horseradish peroxidase-linked goat anti-human kappa
chain antibody was added, incubated for one hour at room
temperature, and washed. Substrate for the horseradish
25 peroxidase was added for detection. Antibody levels in
the COS cell media were found to be nearly undetectable
for the TF8HCDR1 x chimeric LC. Upon closer examination
of the TF8HCDR1 variable region sequence, it was found
that an unexpected base change, which had occurred
30 during the site-directed mutagenesis process described
in Example 3, introduced a stop codon into framework 4

of the TF8HCDR1 gene. This substitution was corrected
1 by site-directed mutagenesis as described above.
Thorough sequencing of the variable region confirmed
that the correction was made with no additional changes
introduced. Upon transfection of this corrected
5 TF8HCDR1 gene with the chimeric LC, reasonable
expression levels were obtained.

COS cells which had been co-transfected with
the CDR-grafted LC expression vector, pSGTF8LCDR1, and
either the chimeric HC or TF8HCDR1, produced antibody at
10 reasonable levels. Antibody levels in COS cell
supernatants ranged from 0.5 μ g to 10.0 μ g per ml.

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EXAMPLE 5

1 Binding of the CDR-Grafted TF8-5G9 to Tissue Factor

 An ELISA was used to determine the ability of
the CDR-grafted TF8-5G9 antibody, TF8HCDR1 x TF8LCDR1,
5 to bind to tissue factor. Tissue factor was immobilized
on a microtiter plate. The test COS cell supernatant,
containing the CDR-grafted antibody, was added to the
well, incubated for one hour at room temperature.
Following three washes with PBS/Tween, a goat anti-human
10 kappa chain polyclonal antibody conjugated to
horseradish peroxidase was added, incubated for one hour
at room temperature and washed. Substrate for the
horseradish peroxidase was added for detection. The
positive control was the TF8-5G9 chimeric antibody. The
15 CDR-grafted TF8-5G9 antibody was able to bind to tissue
factor to a degree comparable to the chimeric TF8-5G9
antibody (Figure 3, solid symbols).

 The ability of the humanized antibody to
compete with murine TF8-5G9 for binding to tissue factor
20 was also examined. Varying amounts of COS cell
supernatant containing the test CDR-grafted antibody and
a fixed amount of murine TF8-5G9 were added
simultaneously to wells coated with tissue factor.
Binding was allowed to occur for one hour at room
25 temperature. The wells were washed three times with
PBS/Tween. A goat anti-human kappa chain antibody
conjugated to horseradish peroxidase was added,
incubated for one hour at room temperature and washed.
Substrate for the horseradish peroxidase was added for
30 detection. The positive antibody competed as well as

the chimeric antibody with murine TF8-5G9 for binding to
1 TF.

These data indicate that the initially
designed CDR-grafted antibody, TF8HCDR1 x TF8LCDR1, was
approximately as active as the chimeric TF8-5G9 in
5 binding to TF and competing with the murine antibody for
binding to TF.

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EXAMPLE 6

1 Construction and Characterization
 of Additional CDR-Grafted Heavy Chains

 Upon examination of the molecular structure of
5 murine TF8-5G9, framework residues at positions 27, 68,
 73 and 78 were found to lie on the antibody surface and
 had no discernible contact with the CDRs. These
 framework residues were of murine sequence in TF8HCDR1
 but were changed to the human KOL sequence in various
10 combinations to generate a series of CDR-grafted heavy
 chains with framework residue variations. The changes
 were made by the process of site-directed mutagenesis as
 described in Example 3. Each CDR-grafted heavy chain
 version was expressed in COS cells in combination with
15 the CDR-grafted LC, TF8LCDR1, and tested for its ability
 to bind TF and compete with murine TF8-5G9 for binding.
 Every version of the CDR-grafted heavy chain in
 combination with TF8LCDR1 was shown to bind TF with an
 affinity comparable to chimeric TF8-5G9. Every CDR-
20 grafted HC in combination with TF8LCDR1 was able to
 compete with murine TF8-5G9 for binding to TF to a
 degree comparable to the chimeric antibody.

 Changes in sequence from murine to human for
 HC framework positions 6, 7, 68, 73 and 78 did not
25 adversely affect the antigen binding ability of the
 antibody. The CDR-grafted HC version which had human
 sequence at all of these positions, and thus was the
 most humanized HC, was TF8HCDR20.

 The complete sequence of the TF8HCDR20 gene
30 was determined. The DNA sequence is shown as a 2360 bp
 EcoRI/BamHI insert with protein translation in the

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pEe6TF8HCDR20 expression vector in Figure 4 and SEQ ID
1 NO:15.

The essential regions of the gene are as
follows:

	Nucleotide #	Region
5	1-6	5' <u>Eco</u> RI restriction site
	7-15	Kozak sequence
	16-72	Start codon and leader sequence
	73-423	CDR-grafted variable region
	424-717	Human IgG4 CH1 domain
10	718-1110	Human IgG4 intron 2
	1111-1146	Human IgG4 hinge
	1147-1267	Human IgG4 intron 3
	1268-1594	Human IgG4 CH2 domain
	1595-1691	Human IgG4 intron 4
15	1692-2012	Human IgG4 CH3 domain
	2013-2354	3' untranslated region
	2355-2360	3' <u>Bam</u> HI end spliced to <u>Bcl</u> I site of the expression vector

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EXAMPLE 7

1 Construction and Characterization
 of Additional CDR-Grafted Light Chains

 The initially designed CDR-grafted LC,
5 TF8LCDR1, contained four framework residues from the
 murine TF8-5G9 sequence. At two of these positions, 39
 and 105, the human REI framework sequence is unique to
 REI; however, the murine TF8-5G9 LC sequence is in
 agreement with the human consensus sequence. The other
10 two murine framework residues, trp41 and thr46, are
 unique to TF8-5G9. Several versions of the CDR-grafted
 LC were generated in which the sequence at these four
 positions were changed from the murine to the human REI
 in various combinations. These changes were made by
15 site-directed mutagenesis. Each version of the CDR-
 grafted LC was expressed in COS cells in combination
 with the CDR-grafted HC, TF8HCDR20, and tested for
 ability to bind tissue factor and compete with murine
 TF8-5G9 for binding. Every version of the CDR-grafted
20 LC, in combination with TF8HCDR20, was shown to bind TF
 with an affinity comparable to TF8-5G9. Also every CDR-
 grafted LC version, in combination with TF8HCDR20, was
 able to compete with murine TF8-5G9 for binding to TF in
 a manner comparable to the chimeric TF8-5G9 control.

25 Changes in sequence from murine to human for
 LC framework positions 39, 41, 46 and 105 did not
 adversely effect the ability of the antibody to
 recognize antigen. The CDR-grafted LC of choice was
 TF8LCDR3, where murine TF8-5G9 sequence was used at
30 positions 39 and 105 because these are in agreement with

the human consensus sequence. The preferred CDR-grafted
 1 TF8-5G9 antibody is TF8HCDR20 x TF8LCDR3.

The complete sequence of the TF8LCDR3 gene was
 determined and is shown as a 759 bp EcoRI-BamHI insert
 with protein translation in the pEel2TF8LCDR3 expression
 5 vector in Figure 5 and SEQ ID NO:17. The essential
 regions of the gene are as follows:

	Nucleotide #	Region
	1-5	5' <u>EcoRI</u> restriction site
	6-8	Kozak sequence
10	9-68	Start codon and leader sequence
	69-392	CDR-grafted variable region
	393-710	Human kappa constant region
	711-753	3' untranslated region
15	754-759	3' <u>BamHI</u> end spliced to <u>BclI</u> site of the expression vector

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EXAMPLE 8

1 CDR-Grafted TF8-5G9 Antibody TF8HCDR20 x TF8LCDR3
 Inhibits Human Tissue Factor

 The binding of the CDR-grafted TF8-5G9
5 antibody, TF8HCDR20 x TF8LCDR3, to TF was assessed as
 described in Example 5 and was found to be comparable to
 that of the chimeric TF8-5G9 as illustrated in Figure 6.
 The ability of the CDR-grafted TF8-5G9 to compete with
 the murine antibody for binding to TF is comparable to
10 that of the chimeric TF8-5G9 as shown in Figure 7.

 An in vitro assay was used to measure the
 level of inhibition of factor X activation by the CDR-
 grafted TF8-5G9 antibody. In this assay, TF forms an
 active proteolytic complex with factor VII. This
15 complex then converts factor X to factor Xa by
 proteolysis. The activated Xa enzymatically cleaves a
 substrate, Spectrozyme FXa, which releases a chromogen.
 The level of chromogen, as detected by optical density,
 is an indication of factor X activation due to TF-factor
20 VIIa activity.

 The following reaction mixtures were prepared
 in 12 x 75 mm borosilicate glass tubes.

 25 μ l TBS (50 mM Tris, pH 7.4, 150 mM NaCl)
 15 μ l 20 mM CaCl₂/1% bovine serum albumin
25 (BSA)
 20 μ l human placental tissue factor solution
 (prepared by reconstituting one vial of
 Thromborel S, Curtin Matheson Scientific
 #269-338 with 4.0 ml dH₂O and diluting
30 1:10 in TBS)

30 μ l Factor VII (Enzyme Research Labs #HFVII
1 1007 at 237.66 ng/ml in TBS)
30 μ l TBS or TF8-5G9 or TF8MCDR20 x TF8LCDR3
at 1.18 μ g/ml or as indicated in Fig. 8
The reaction mixtures were incubated at 37°C
5 for ten minutes before the addition of Factor X. (In
some cases the reaction mixture was preincubated for
five minutes before addition of Factor VII or antibody,
followed by a ten minute incubation before addition of
Factor X.) Thirty μ l of Factor X solution (Enzyme
10 Research Labs, DHFX 330, 247.38 μ g/ml TBS) was added and
the mixture was incubated at 37°C for three minutes.
Factor X activation was terminated by pipetting 40 μ g of
reaction mixture into 160 μ l of stop buffer (50 mM Tris,
pH 7.4, 100 mM EDTA, 150 mM NaCl) in 96 well microtiter
15 plates. Each tube of reaction mixture was pipetted into
three microtiter wells. Fifty μ l of Spectrozyme FXa
substrate (American Diagnostica #222, 1 μ M/ml TBS) was
added to each well. OD₄₀₅ was read on a Molecular
Devices kinetic plate reader with readings taken every
20 twenty seconds for ten minutes. Factor X activity was
recorded as mOD/minute, and enzyme velocities over the
linear portion of the reaction curve were compared to
determine inhibition of factor X activation by the anti-
TF antibodies.

25 As shown in Figure 8, the CDR-grafted TF8-5G9
antibody is approximately as effective as the murine
TF8-5G9 in inhibiting factor X activation. This
indicates that the CDR-grafted TF8-5G9 is functionally
active.

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EXAMPLE 9

1 Construction of the CDR-Grafted Heavy
 and Light Chain Myeloma Expression Vectors

 For the purpose of establishing a permanent
5 CDR-grafted antibody-producing cell line, the TF8HCDR20
and TF8LCDR3 genes were subcloned into myeloma cell
expression vectors. The heavy chain TF8HCDR20 was
subcloned into the EcoRI and BclI sites of the pEe6hCMV-
BglII myeloma expression vector described by Stephens et
10 al. (1989) Nucleic Acids Res. 17:7110 to produce
pEe6TF8HCDR20. The light chain TF8LCDR3 was subcloned
into the EcoTI and BclI sites of the pEel2 myeloma
expression vector to produce pEel2TF8LCDR3. The heavy
and light chain expression vectors are illustrated in
15 Figures 9 and 10, respectively. In both vectors
antibody gene transcription was driven by the human
cytomegalovirus (hCMV) promoter-enhancer, which lies
directly 5' to the multiple cloning site. The
polyadenylation signal sequence lies 3' to the multiple
20 cloning site and signals the termination of
transcription. Each vector contains the β -lactamase
gene to allow for ampicillin selection in E. coli. The
pEel2 vector contains a glutamine synthetase cDNA gene
under the transcriptional control of the SV40 early
25 promoter. Glutamine synthetase allows for myeloma cell
transfectants to be selected in glutamine-free media.
Myeloma cells are devoid of glutamine synthetase
activity and are dependent on a supply of glutamine in
the culture media. Cells which have been transfected
30 with the pEel2 vector, containing the glutamine

synthetase gene, are able to synthesize glutamine from
1 glutamate and can survive in the absence of glutamine.

The pEe6TF8HCDR20 expression vector is a 7073 bp
plasmid whose DNA sequence is shown in Figure 4 and SEQ
ID NO:15. The coding regions of the TF8HCDR20 gene are
5 translated. The essential regions of this vector are
described below:

1. Nucleotides #1-2360: The TF8HCDR20 CDR-
grafted HC gene is described in Example
6. The HC gene was inserted as an
10 EcoRI/BamHI fragment into the EcoRI/BclI
sites of the pEe6hCMV-BglII vector.
2. Nucleotides #2361-2593: This region
encodes the SV40 early gene
polyadenylation signal (SV40 nucleotides
2770-2537), which acts as a
transcriptional terminator. This
15 fragment is flanked by a 5' BclI site and
a 3' BamHI site. The 3' BamHI end of the
heavy chain gene was spliced to the 5'
BclI site of the polyadenylation signal,
thus eliminating both sites.
3. Nucleotides #2594-3848: This region is a
BamHI-BglI fragment from pBR328
20 (nucleotides 375-2422) but with a
deletion between the SalI and AvaI sites
(pBR328 nucleotides 651-1425) following
the addition of a SalI linker to the AvaI
site. This region contains the Col E1
bacterial origin of replication.
- 25 4. Nucleotides #3849-4327: This is a BglI-
XmnI fragment site from the β -lactamase
gene of pSP64 (Promega Corporation,
Madison, WI). This gene provides
ampicillin resistance to bacteria
transformed with this vector.
- 30 5. Nucleotides #4328-4885: This is an XmnI-
HindIII fragment of the ColE1 based
plasmid pCT54 described by Emtage et al.
(1983) Proc. Natl. Acad. Sci. USA

1 80:3671. The HindIII site was converted
to a BglII site by the addition of a
linker following the addition of the hCMV
promoter described below.

- 5 6. Nucleotides #4886-7022: These
nucleotides encode the Pst-1m fragment of
human cytomeglovirus (hCMV) strain AD 169
described by Greenway et al. (1982) Gene
18:355 containing the region coding for
the hCMV middle intermediate early
promoter. This Pst-1m fragment was
cloned into the HindIII site of pEe6hCMV
by addition of oligonucleotides of the
10 following sequence to either end of the
fragment:

5' GTCACCGTCCTTGACACGA 3'

3' ACGTCAGTGGCAGGAAGTGTGCTTCGA 5'

15 The resulting 2100 bp fragment was
inserted such that the promoter directed
transcription towards the EcoRI site of
pEe6hCMV. The oligonucleotide above
served to recreate the complete 5'
untranslated sequence of the hCMV-MIE
gene the added irrelevant sequence at the
very 5' end of the fragment. The HindIII
20 site at the 5' end was subsequently
converted to a BglII site by the addition
of a further linker.

7. Nucleotides #7023-7073: The pSP64
polylinker with the BamHI and SaII sites
removed.

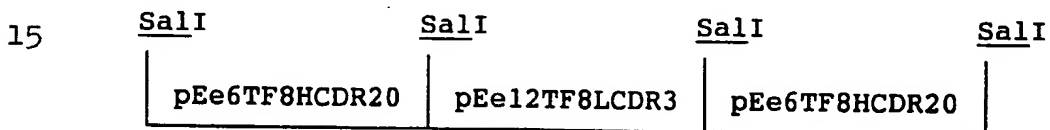
25 The pEe12TF8LCDR3 expression vector is a 7864
bp plasmid whose DNA sequence is shown in Figure 5 and
SEQ ID NO:17. The coding regions of the TF8LCDR3 gene
are translated. The essential regions of this
expression vector are described below:

- 30 1. Nucleotides #1-759: The TF8LCDR3 CDR-
grafted LC gene is described in Example
7. The gene was inserted as an

- 1 EcoRI/BamHI fragment into the EcoRI/BclII
sites of the pEel2 expression vector.
2. Nucleotides #760-3284: These regions of
pEel2 are identical to the regions
encoded by nucleotides 2361-4885 of the
pEe6TF8HCDR20 vector described above
5 (regions #2-5).
3. Nucleotides #3285-5736: This region
encodes the Chinese hamster ovary
glutamine synthetase cDNA under the
transcriptional control of the SV40 early
promoter and followed by the SV40
polyadenylation and splice signals from
the pSV2.dhfr vector described by
10 Subramani et al. (1981) Mol. Cell. Biol.
1:854. The following describes the
derivation of this region: A 1200 bp
NaeI-PvuII fragment, containing a
complete GS coding sequence, was excised
from the Chinese hamster ovary cDNA clone
λGS1.1 described by Hayward et al. (1986)
15 Nucleic Acid Res. 14:999. After addition
of a HindIII linker to the NaeI site and
a BglII linker to the PvuII site (hence
destroying the NaeI and PvuII sites), the
1200 bp fragment was cloned in place of
DHFR sequences in pSV2.dhfr between the
20 HindIII and BglII sites to form pSV2.GS.
The single remaining PvuII site in
pSV2BamGS was converted to a BamHI site
by addition of an oligonucleotide linker
to form pSV2BamGS. An EcoRI site in the
GS cDNA was destroyed by site directed
mutagenesis without altering the amino
acid sequence in pSV2BamGS and the
25 HindIII site was destroyed by filling in
with DNA polymerase I. The 2451 bp BamHI
fragment from this plasmid, containing
the complete SV40-GS hybrid transcription
unit, was excised and inserted at the
BglII site of pEe6hCMV-BglII site of
pEe6hCMV-BglII such that transcription
30 from the SV40 early promoter proceeds
towards the hCMV promoter.

4. Nucleotides #5737-7864: This region is identical to the hCMV promoter and pSP64 polylinker encoded by nucleotides 4886-7073 of the pEe6TF8HCDR20 vector described above (regions 6 and 7).

For the purpose of ensuring that both the pEe6TF8HCDR20 and pEe12TF8LCDR3 vectors co-transfected myeloma cells, the vectors were joined in linear concatamers. Both the pEe6TF8HCDR20 and pEe12TF8LCDR3 vectors were digested at the unique SalI site. The SalI linearized pEe6TF8HCDR20 vector was phosphatased at its 5' ends to prohibit ligation of two pEe6TF8HCDR20 vectors onto each other. This phosphatased HC vector was ligated in a 2:1 molar ratio to the SalI linearized pEe12TF8LCDR3. The resulting concatamers were most likely of the following composition:



This concatamerized DNA was extracted with phenol and chloroform, and precipitated with ammonium acetate and ethanol. The DNA precipitate was resuspended in distilled water to a concentration of 1 $\mu\text{g}/\mu\text{L}$ and used to transfect myeloma cells.

EXAMPLE 10

1 Development of NSO Expression Cell Lines

Stably transformed cell lines expressing the humanized TF8-5G9 antibody were prepared by transfecting
5 CDR-grafted heavy and light chain expression vectors into NSO mouse myeloma cells. Selection of transfected cells was carried out using the dominant selectable marker gene, glutamine synthetase (GS).

The NSO mouse myeloma cell line, obtained from
10 Celltech, Ltd., is a subclone derived from NS-1 and does not express intracellular light chains. These cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with added glutamine and 10% fetal bovine serum (FBS). To prepare for transfection, the cells were
15 harvested in mid-log phase of the growth cycle, centrifuged for 5 minutes, washed with phosphate buffered saline (PBS), centrifuged again, and the cell pellet was resuspended in 2.2 mL of PBS. The final cell concentration was 2.18×10^7 mL. Cells were maintained
20 on ice during the entire procedure.

The DNA to be transfected (pEel2TF8LCDR3 x pEe6TF8HCDR20) was prepared as a concatamer as described in Example 9. The DNA and NSO cells were added to a 0.4 cm BioRad Gene Pulser cuvette in the following order:

25 40 μ L (40 μ g) DNA concatamer
 320 μ L double distilled water
 40 μ L 10 x PBS
 400 μ L NSO cells (8.72×10^6 cells)

Transfection was performed by electroporation
30 following a protocol provided by Celltech, Ltd. In this procedure, the cells and DNA in PBS buffer were exposed

to a brief, high voltage pulse of electricity causing
1 transient micropores to form on the cell membrane. DNA
transfer takes place through these openings. To prepare
for electroporation, the suspension of NSO cells and DNA
was gently mixed and incubated on ice for 5 minutes.

5 The cuvette was placed in a BioRad Gene Pulser and given
2 consecutive electrical pulses at settings of 3 μ F
(capacitance) and 1.5V (voltage). Following
electroporation, the cuvette was returned to the ice for
5 minutes. The suspension was then diluted in prewarmed
10 growth medium and distributed into seven 96-well plates.
Control plates containing cells electroporated without
DNA were also prepared at the same time to measure the
presence of spontaneous mutants. Plates were placed in
a 37°C incubator with 5% CO₂.

15 Glutamine synthetase, encoded by the GS gene,
is an enzyme that converts glutamate to glutamine. NSO
cells require glutamine for growth due to inadequate
levels of endogenous GS gene expression. In the DNA
concatamer, this gene is located on the pEel2TF8LCDR3
20 vector. Transfected cells which incorporate the GS gene
become glutamine-independent. Cells not integrating the
GS gene into their genome would remain glutamine-
dependent and would not survive in glutamine-free
medium. Approximately 18 hours post electroporation,
25 all plates were fed with glutamine-free selection medium
and returned to the incubator until viable colonies
appeared.

Approximately 3 weeks after transfection,
distinct macroscopic colonies were observed. These were
30 screened for expression of the intact humanized antibody
using the assembly ELISA as described in Example 5.

Tissue culture supernatants from wells containing colonies were screened at a 1:10 dilution. Positive wells showing activity greater than the 25 ng/mL standard were subcultured and expanded for further analysis.

For selection of high producers, antibody production was quantitated after a 96 hour growth period. Tissue culture flasks were seeded with 2×10^5 cells/mL in 10 mL of selection medium and incubated at 37°C, 5% CO₂ for 96 hours. At the end of that time period, an aliquot was taken to determine cell concentration and antibody titer. Evaluation of antibody production was calculated as µg/mL and pg/cell/96 hours. The highest producers from this transfection were:

<u>Cell Line</u>	<u>µg/mL</u>	<u>pg/cell/96 hour</u>
2B1	26.3	24.3
3E11	27.6	59.9
4G6	30.2	41.9

EXAMPLE 11

1 CDR Grafted Antibody TF8HCDR20 x TF8LCDR3
 Inhibits Tissue Factor In Vivo

5 CDR grafted antibody TF8HCDR20 x TF8LCDR3 was
6 compared to murine antibody TF8-5G9 for its ability to
7 protect rats from experimentally induced disseminated
8 intravascular coagulation (DIC). In the DIC model, rats
9 are challenged with human thromboplastin (a crude tissue
10 extract containing TF activity), resulting in fibrinogen
11 consumption and death. Pretreatment of rats with anti-
12 TF antibody was demonstrated to protect rats from
13 fibrinogen consumption and death as follows.

14 Human thromboplastin was prepared as described
15 in U.S. Patent 5,223,427. Saline control or 30 μ /ml of
16 TF8-5G9 or CDR-grafted antibody was injected through the
17 tail vein of rats, followed by injection of
18 thromboplastin equivalent to 200 ng of recombinant TF.
19 Clotting times were determined at T=0 and T=1 minute as
20 a measure of fibrinogen concentration. Clotting times
21 are proportional to fibrinogen concentration, with a 60
22 second clotting time corresponding to an 80% reduction
23 in fibrinogen concentration. Clotting times of greater
24 than 60 seconds cannot be accurately measured and were
25 recorded as 60 seconds.

26 Survivability and clotting times for three
27 representative studies are shown below.

<u>Survivors</u>			
Study	Controls	TF8-5G9	CDR-grafted Ab
30 1	0/8	5/8	6/8
2	0/8	4/7	7/8
3	0/8	8/8	3/7

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		<u>Clotting Times</u>					
		<u>Controls</u>					
1	Study #1		Study #2		Study #3		
	<u>T=0</u>	<u>T=1</u>	<u>T=0</u>	<u>T=1</u>	<u>T=0</u>	<u>T=1</u>	
5	16	>60	18	>60	19	>60	
	16	>60	18	>60	21	>60	
	16	>60	18	>60	18	>60	
	17	>60	18	>60	19	>60	
	15	>60	16	>60	18	54	
	16	>60	18	>60	18	>60	
	16	>60	17	>60	18	>60	
	16	>60	17	>60	18	>60	

		<u>Clotting Times</u>					
		<u>Murine TF8-5G9</u>					
10	Study #1		Study #2		Study #3		
	<u>T=0</u>	<u>T=1</u>	<u>T=0</u>	<u>T=1</u>	<u>T=0</u>	<u>T=1</u>	
15	16	36	18	34	19	28	
	15	41	18	36	18	29	
	15	33	18	>60	19	29	
	15	31	17	>60	18	29	
	15	>60	18	50	18	28	
	16	>60	17	34	19	40	
	16	33	17	34	19	40	
	16	33	18	31	19	34	
20	16	>60			19	>60	

		<u>Clotting Times</u>					
		<u>CDR-grafted TF8-5G9</u>					
25	Study #1		Study #2		Study #3		
	<u>T=0</u>	<u>T=1</u>	<u>T=0</u>	<u>T=1</u>	<u>T=0</u>	<u>T=1</u>	
30	16	>60	17	>60	21	>60	
	16	>60	17	33	18	34	
	16	>60	18	32	17	>60	
	22	37	18	>60	20	35	
	16	32	17	32	17	58	
	15	>60	18	31	18	33	
	16	>60	17	31	18	31	
	16	>60	16	32			

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Twenty-three of the twenty-four control rats
1 had clotting times of greater than 60 seconds indicating
that virtually all untreated rats were consuming more
than 80% of their fibrinogen. Both the CDR-grafted and
murine antibody treated rats had similar clotting times
5 at one minute of 44.5 and 40 seconds. Further, only six
of the murine antibody treated rats and nine of the CDR-
grafted antibody treated rats had clotting times in
excess of 60 seconds. Accordingly, both the murine and
CDR-grafted antibodies were able to neutralize TF and
10 thus protect rats from fibrinogen consumption and death.

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SEQUENCE LISTING

1

(1) GENERAL INFORMATION:

(i) APPLICANT: Joliffe, Linda K.
Zivin, Robert A.
Pulito, Virginia L.

5

(ii) TITLE OF INVENTION: CDR-GRAFTED ANTI-TISSUE FACTOR
ANTIBODIES AND METHODS OF USE THEREOF

(iii) NUMBER OF SEQUENCES: 20

(iv) CORRESPONDENCE ADDRESS:

10

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(v) COMPUTER READABLE FORM:

15

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE: 07-JUN-1995
(C) CLASSIFICATION:

20

(viii)- ATTORNEY/AGENT INFORMATION:

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(2) INFORMATION FOR SEQ ID NO:1:

1 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1489 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 11..1391

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

10	GGTCCTTACA ATG AAA TGC AGC TGG GTC ATC TTC TTC CTG ATG GCA GTG	49
	Met Lys Cys Ser Trp Val Ile Phe Phe Leu Met Ala Val	
	1 5 10	
	GTT ACA GGG GTC AAT TCA GAG ATT CAG CTG CAG CAG TCT GGG GCT GAG	97
	Val Thr Gly Val Asn Ser Glu Ile Gln Leu Gln Gln Ser Gly Ala Glu	
	15 20 25	
15	CTT GTG AGG CCA GGG GCC TTA GTC AAG TTG TCC TGC AAA GCT TCT GGC	145
	Leu Val Arg Pro Gly Ala Leu Val Lys Leu Ser Cys Lys Ala Ser Gly	
	30 35 40 45	
	TTC AAC ATT AAA GAC TAC TAT ATG CAC TGG GTG AAG CAG AGG CCT GAA	193
	Phe Asn Ile Lys Asp Tyr Tyr Met His Trp Val Lys Gln Arg Pro Glu	
	50 55 60	
	CAG GGC CTG GAG TGG ATT GGA TTG ATT GAT CCT GAG AAT GGT AAT ACT	241
	Gln Gly Leu Glu Trp Ile Gly Leu Ile Asp Pro Glu Asn Gly Asn Thr	
	65 70 75	
20	ATA TAT GAC CCG AAG TTC CAG GGC AAG GCC AGT ATA ACA GCA GAC ACA	289
	Ile Tyr Asp Pro Lys Phe Gln Gly Lys Ala Ser Ile Thr Ala Asp Thr	
	80 85 90	
	TCC TCC AAC ACA GCC TAC CTG CAG CTC AGC AGC CTG ACA TCT GAG GAC	337
	Ser Ser Asn Thr Ala Tyr Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp	
	95 100 105	
25	ACT GCC GTC TAT TAC TGT GCT AGA GAT AAC TCG TAC TAC TTT GAC TAC	385
	Thr Ala Val Tyr Tyr Cys Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr	
	110 115 120 125	

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-61-

1	TGG GGC CAA GGC ACC ACT CTC ACA GTC TCC TCA GCC AAA ACG ACA CCC Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Pro	433
	130 135 140	
	CCA TCT GTC TAT CCA CTG GCC CCT GGA TCT GCT GCC CAA ACT AAC TCC Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser Ala Ala Gln Thr Asn Ser	481
	145 150 155	
5	ATG GTG ACC CTG GGA TGC CTG GTC AAG GGC TAT TTC CCT GAG CCA GTG Met Val Thr Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val	529
	160 165 170	
	ACA GTG ACC TGG AAC TCT GGA TCC CTG TCC AGC GGT GTG CAC ACC TTC Thr Val Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe	577
	175 180 185	
10	CCA GCT GTC CTG CAG TCT GAC CTC TAC ACT CTG AGC AGC TCA GTG ACT Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr	625
	190 195 200 205	
	GTG CCC TCC AGC ACC TGG CCC AGC GAG ACC GTC ACC TGC AAC GTT GCC Val Pro Ser Ser Thr Trp Pro Ser Glu Thr Val Thr Cys Asn Val Ala	673
	210 215 220	
	CAC CCG GCC AGC AGC ACC AAG GTG GAC AAG AAA ATT GTG CCC AGG GAT His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys Ile Val Pro Arg Asp	721
	225 230 235	
15	TGT GGT TGT AAG CCT TGC ATA TGT ACA GTC CCA GAA GTA TCA TCT GTC Cys Gly Cys Lys Pro Cys Ile Cys Thr Val Pro Glu Val Ser Ser Val	769
	240 245 250	
	TTC ATC TTC CCC CCA AAG CCC AAG GAT GTG CTC ACC ATT ACT CTG ACT Phe Ile Phe Pro Pro Lys Pro Lys Asp Val Leu Thr Ile Thr Leu Thr	817
	255 260 265	
20	CCT AAG GTC ACG TGT GTT GTG GTA GAC ATC AGC AAG GAT GAT CCC GAG Pro Lys Val Thr Cys Val Val Val Asp Ile Ser Lys Asp Asp Pro Glu	865
	270 275 280 285	
	GTC CAG TTC AGC TGG TTT GTA GAT GAT GTG GAG GTG CAC ACA GCT CAG Val Gln Phe Ser Trp Phe Val Asp Asp Val Glu Val His Thr Ala Gln	913
	290 295 300	
25	ACG CAA CCC CGG GAG GAG CAG TTC AAC AGC ACT TTC CGC TCA GTC AGT Thr Gln Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Ser Val Ser	961
	305 310 315	

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-62-

1	GAA CTT CCC ATC ATG CAC CAG GAC TGG CTC AAT GGC AAG GAG TTC AAA Glu Leu Pro Ile Met His Gln Asp Trp Leu Asn Gly Lys Glu Phe Lys 320 325 330	11009
	TGC AGG GTC AAC AGT GCA GCT TTC CCT GCC CCC ATC GAG AAA ACC ATC Cys Arg Val Asn Ser Ala Phe Pro Ala Pro Ile Glu Lys Thr Ile 335 340 345	11057
5	TCC AAA ACC AAA GGC AGA CCG AAG GCT CCA CAG GTG TAC ACC ATT CCA Ser Lys Thr Lys Gly Arg Pro Lys Ala Pro Gln Val Tyr Thr Ile Pro 350 355 360 365	11105
	CCT CCC AAG GAG CAG ATG GCC AAG GAT AAA GTC AGT CTG AAC TGC ATG Pro Pro Lys Glu Gln Met Ala Lys Asp Lys Val Ser Leu Asn Cys Met 370 375 380	11153
10	ATA ACA GAC TTC TTC CCT GAA GAC ATT ACT GTG GAG TGG CAG TGG AAT Ile Thr Asp Phe Phe Pro Glu Asp Ile Thr Val Glu Trp Gln Trp Asn 385 390 395	11201
	GGG CAG CCA GCG GAG AAC TAC AAG AAC ACT CAG CCC ATC ATG GAC ACA Gly Gln Pro Ala Glu Asn Tyr Lys Asn Thr Gln Pro Ile Met Asp Thr 400 405 410	11249
	GAT GGC TCT TAC TTC GTC TAC AGC AAG CTC AAT GTG CAG AAG AGC AAC Asp Gly Ser Tyr Phe Val Tyr Ser Lys Leu Asn Val Gln Lys Ser Asn 415 420 425	11297
15	TGG GAG GCA GGA AAT ACT TTC ACC TGC TCT GTG TTA CAT GAG GGC CTG Trp Glu Ala Gly Asn Thr Phe Thr Cys Ser Val Leu His Glu Gly Leu 430 435 440 445	11345
	CAC AAC CAC CAT ACT GAG AAG AGC CTC TCC CAC TCT CCT GGT AAA T His Asn His His Thr Glu Lys Ser Leu Ser His Ser Pro Gly Lys 450 455 460	11391
20	GATCCCGAGTG TCCTTGGAGC CCTCTGGTCC TACAGGACTC TGACACCTAC CTCCACCCCT CCCTGTATAA ATAAAGCACC CAGCACTGCC TTGGACCC	11451 11489

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- 25 (A) LENGTH: 460 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: protein

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Lys Cys Ser Trp Val Ile Phe Phe Leu Met Ala Val Val Thr Gly
 1 5 10 15
 Val Asn Ser Glu Ile Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg
 20 25 30
 Pro Gly Ala Leu Val Lys Leu Ser Cys Lys Ala Ser Gly Phe Asn Ile
 35 40 45
 Lys Asp Tyr Tyr Met His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu
 50 55 60
 Glu Trp Ile Gly Leu Ile Asp Pro Glu Asn Gly Asn Thr Ile Tyr Asp
 65 70 75 80
 Pro Lys Phe Gln Gly Lys Ala Ser Ile Thr Ala Asp Thr Ser Ser Asn
 85 90 95
 Thr Ala Tyr Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val
 100 105 110
 Tyr Tyr Cys Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr Trp Gly Gln
 115 120 125
 Gly Thr Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Pro Pro Ser Val
 130 135 140
 Tyr Pro Leu Ala Pro Gly Ser Ala Ala Gln Thr Asn Ser Met Val Thr
 145 150 155 160
 Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val Thr Val Thr
 165 170 175
 Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro Ala Val
 180 185 190
 Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Pro Ser
 195 200 205
 Ser Thr Trp Pro Ser Glu Thr Val Thr Cys Asn Val Ala His Pro Ala
 210 215 220

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1 Ser Ser Thr Lys Val Asp Lys Lys Ile Val Pro Arg Asp Cys Gly Cys
 225 230 235 240
 Lys Pro Cys Ile Cys Thr Val Pro Glu Val Ser Ser Val Phe Ile Phe
 245 250 255
 Pro Pro Lys Pro Lys Asp Val Leu Thr Ile Thr Leu Thr Pro Lys Val
 260 265 270
 5 Thr Cys Val Val Val Asp Ile Ser Lys Asp Asp Pro Glu Val Gln Phe
 275 280 285
 Ser Trp Phe Val Asp Asp Val Glu Val His Thr Ala Gln Thr Gln Pro
 290 295 300
 Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Ser Val Ser Glu Leu Pro
 305 310 315 320
 10 Ile Met His Gln Asp Trp Leu Asn Gly Lys Glu Phe Lys Cys Arg Val
 325 330 335
 Asn Ser Ala Ala Phe Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr
 340 345 350
 Lys Gly Arg Pro Lys Ala Pro Gln Val Tyr Thr Ile Pro Pro Pro Lys
 355 360 365
 15 Glu Gln Met Ala Lys Asp Lys Val Ser Leu Asn Cys Met Ile Thr Asp
 370 375 380
 Phe Phe Pro Glu Asp Ile Thr Val Glu Trp Gln Trp Asn Gly Gln Pro
 385 390 395 400
 Ala Glu Asn Tyr Lys Asn Thr Gln Pro Ile Met Asp Thr Asp Gly Ser
 405 410 415
 20 Tyr Phe Val Tyr Ser Lys Leu Asn Val Gln Lys Ser Asn Trp Glu Ala
 420 425 430
 Gly Asn Thr Phe Thr Cys Ser Val Leu His Glu Gly Leu His Asn His
 435 440 445
 His Thr Glu Lys Ser Leu Ser His Ser Pro Gly Lys
 450 455 460

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(2) INFORMATION FOR SEQ ID NO:3:

- 1 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 937 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

- 5 (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 5..706

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

10	GGAC ATG CGG GCC CCT GCT CAG TTT TTT GGG ATC TTG TTG CTC TGG TTT Met Arg Ala Pro Ala Gln Phe Phe Gly Ile Leu Leu Leu Trp Phe	49
	1 5 10 15	
	CCA GGT ATC AGA TGT GAC ATC AAG ATG ACC CAG TCT CCA TCC TCC ATG Pro Gly Ile Arg Cys Asp Ile Lys Met Thr Gln Ser Pro Ser Ser Met	97
	20 25 30	
15	TAT GCA TCG CTG GGA GAG AGA GTC ACT ATC ACT TGT AAG GCG AGT CAG Tyr Ala Ser Leu Gly Glu Arg Val Thr Ile Thr Cys Lys Ala Ser Gln	145
	35 40 45	
	GAC ATT AGA AAG TAT TTA AAC TGG TAC CAG CAG AAA CCA TGG AAA TCT Asp Ile Arg Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Trp Lys Ser	193
	50 55 60	
	CCT AAG ACC CTG ATC TAT TAT GCA ACA AGC TTG GCA GAT GGG GTC CCA Pro Lys Thr Leu Ile Tyr Tyr Ala Thr Ser Leu Ala Asp Gly Val Pro	241
20	65 70 75	
	TCA AGA TTC AGT GGC AGT GGA TCT GGG CAA GAT TAT TCT CTA ACC ATC Ser Arg Phe Ser Gly Ser Gly Ser Gly Gln Asp Tyr Ser Leu Thr Ile	289
	80 85 90 95	
	AGC AGC CTG GAG TCT GAC GAT ACA GCA ACT TAT TAC TGT CTA CAA CAT Ser Ser Leu Glu Ser Asp Asp Thr Ala Thr Tyr Tyr Cys Leu Gln His	337
	100 105 110	
25	GGT GAG AGC CCG TAC ACG TTC GGA GGG GGG ACC AAG CTG GAA ATA AAC Gly Glu Ser Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Asn	385
	115 120 125	

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1	AGG GCT GAT GCT GCA CCA ACT GTA TCC ATC TTC CCA CCA TCC AGT GAG Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu 130 135 140	433
	CAG TTA ACA TCT GGA GGT GCC TCA GTC GTG TGC TTC TTG AAC AAC TTC Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe 145 150 155	481
5	TAC CCC AAA GAC ATC AAT GTC AAG TGG AAG ATT GAT GGC AGT GAA CGA Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg 160 165 170 175	529
	CAA AAT GGC GTC CTG AAC AGT TGG ACT GAT CAG GAC AGC AAA GAC AGC Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser 180 185 190	577
10	ACC TAC AGC ATG AGC AGC ACC CTC ACG TTG ACC AAG GAC GAG TAT GAA Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu 195 200 205	625
	CGA CAT AAC AGC TAT ACC TGT GAG GCC ACT CAC AAG ACA TCA ACT TCA Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser 210 215 220	673
15	CCC AAT GTC AAG AGC TTC AAC AAG AAT GAG TGT TAGAGACAAA GGTCCTGAGA Pro Asn Val Lys Ser Phe Asn Lys Asn Glu Cys 225 230	726
	CGCCACCACC AGCTCCCCAG CTCCATCCTA TCTTCCCTTC TAAGGTCTTG GAGGCTTCCC	786
	CACAAGCGAC CTACCACTGT TGCGGTGCTC CAAACCTCCT CCCACCTCC TTCTCCTCCT	846
	CCTCCCTTTC CTTGGCTTTT ATCATGCTAA TATTGTCAGA AAATATTCAA TAAAGTGAGT	906
	CTTTGCACTT GAAAAAAAAA AAAAAAAAAA A	937

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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 234 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: protein

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

1 Met Arg Ala Pro Ala Gln Phe Phe Gly Ile Leu Leu Leu Trp Phe Pro
 1 5 10 15
 Gly Ile Arg Cys Asp Ile Lys Met Thr Gln Ser Pro Ser Ser Met Tyr
 20 25 30
 5 Ala Ser Leu Gly Glu Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp
 35 40 45
 Ile Arg Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Trp Lys Ser Pro
 50 55 60
 Lys Thr Leu Ile Tyr Tyr Ala Thr Ser Leu Ala Asp Gly Val Pro Ser
 65 70 75 80
 10 Arg Phe Ser Gly Ser Gly Ser Gly Gln Asp Tyr Ser Leu Thr Ile Ser
 85 90 95
 Ser Leu Glu Ser Asp Asp Thr Ala Thr Tyr Tyr Cys Leu Gln His Gly
 100 105 110
 Glu Ser Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Asn Arg
 115 120 125
 15 Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln
 130 135 140
 Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr
 145 150 155 160
 Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln
 165 170 175
 20 Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr
 180 185 190
 Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg
 195 200 205
 His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro
 210 215 220
 25 Asn Val Lys Ser Phe Asn Lys Asn Glu Cys
 225 230

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(2) INFORMATION FOR SEQ ID NO:5:

- 1 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- 5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:
Asp Asp Tyr Met His
1 5

10 (2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- 15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:
Leu Ile Asp Pro Glu Asn Gly Asn Thr Ile Tyr Lys Pro Lys Phe Gln
1 5 10 15
Gly

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(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
- 25 (ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

1 Asp Asn Ser Tyr Tyr Phe Asp Tyr
 1 5

(2) INFORMATION FOR SEQ ID NO:8:

5 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 11 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

 Lys Ala Ser Gln Asp Ile Arg Lys Tyr Leu Asn
 1 5 10

(2) INFORMATION FOR SEQ ID NO:9:

15 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 7 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

 Tyr Ala Thr Ser Leu Ala Asp
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(2) INFORMATION FOR SEQ ID NO:10:

- 1 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 9 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- 5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:
- Leu Gln His Gly Glu Ser Pro Tyr Thr
 1 5

10 (2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 117 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- 15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:
- Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg
 1 5 10 15
- Leu Leu Arg Leu Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys Asp Tyr
 20 25 30
- Tyr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
 35 40 45
- Gly Leu Ile Asp Pro Glu Asn Gly Asn Thr Ile Tyr Asp Pro Lys Phe
 50 55 60
- Gln Gly Arg Phe Ser Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Phe
 25 65 70 75 80
- 30
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1 Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Pro
100 105 110

Val Thr Val Ser Ser
115

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(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 108 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15

15 Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Ile Arg Lys Tyr
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Trp Lys Ala Pro Lys Thr Leu Ile
35 40 45

Tyr Tyr Ala Thr Ser Leu Ala Asp Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

20 Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Ile Ala Thr Tyr Tyr Cys Leu Gln His Gly Glu Ser Pro Tyr
85 90 95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Thr Arg
100 105

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(2) INFORMATION FOR SEQ ID NO:13:

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(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 117 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

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Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg
1 5 10 15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys Asp Tyr
20 25 30

Tyr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45

Gly Leu Ile Asp Pro Glu Asn Gly Asn Thr Ile Tyr Asp Pro Lys Phe
50 55 60

Gln Gly Arg Phe Thr Ile Ser Ala Asp Asn Ser Lys Asn Thr Leu Phe
65 70 75 80

15

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Pro
100 105 110

Val Thr Val Ser Ser
115

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(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 108 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1 5 10 15
 Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Ile Arg Lys Tyr
 20 25 30
 Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
 35 40 45
 Tyr Tyr Ala Thr Ser Leu Ala Asp Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60
 Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro
 65 70 75 80
 Glu Asp Ile Ala Thr Tyr Tyr Cys Leu Gln His Gly Glu Ser Pro Tyr
 85 90 95
 Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Thr Arg
 100 105

15

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 7073 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: CDS
 (B) LOCATION: 61..717

(ix) FEATURE:

(A) NAME/KEY: CDS
 (B) LOCATION: 1111..1146

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(ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1268..1594

(ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1692..2012

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

	GAATTCGCCT CCACCATGGA ATGGAGCTGG GTCTTTCTCT TCTTCTTGTC AGTAACTACA	60
	GGT GTA CAC TCA CAA GTT CAG CTG GTG GAG TCT GGA GGA GGA GTA GTA	108
	Gly Val His Ser Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val	
	1 5 10 15	
10	CAA CCT GGA AGG TCA CTG AGA CTG TCT TGT AAG GCT AGT GGA TTC AAT	156
	Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Phe Asn	
	20 25 30	
	ATC AAG GAC TAT TAT ATG CAC TGG GTC AGA CAA GCT CCT GGA AAA GGA	204
	Ile Lys Asp Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly	
	35 40 45	
15	CTC GAG TGG ATA GGT TTA ATT GAT CCT GAG AAT GGT AAC ACG ATA TAT	252
	Leu Glu Trp Ile Gly Leu Ile Asp Pro Glu Asn Gly Asn Thr Ile Tyr	
	50 55 60	
	GAT CCC AAG TTC CAA GGA AGA TTC ATA ATT TCT GCA GAC AAC TCT AAG	300
	Asp Pro Lys Phe Gln Gly Arg Phe Ile Ile Ser Ala Asp Asn Ser Lys	
	65 70 75 80	
	AAT ACA CTG TTC CTG CAG ATG GAC TCA CTC AGA CCT GAG GAT ACA GCA	348
	Asn Thr Leu Phe Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala	
	85 90 95	
20	GTC TAC TTT TGT GCT AGA GAT AAC AGT TAT TAC TTC GAC TAC TGG GGC	396
	Val Tyr Phe Cys Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr Trp Gly	
	100 105 110	
	CAA GGA ACA CCA GTC ACC GTG AGC TCA GCT TCC ACC AAG GGC CCA TCC	444
	Gln Gly Thr Pro Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser	
	115 120 125	
25	GTC TTC CCC CTG GCG CCC TGC TCC AGG AGC ACC TCC GAG AGC ACA GCC	492
	Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala	
	130 135 140	

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1	GCC CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val 145 150 155 160	540
	TCG TGG AAC TCA GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala 165 170 175	588
5	GTC CTA CAG TCC TCA GGA CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val 180 185 190	636
	CCC TCC AGC AGC TTG GGC ACG AAG ACC TAC ACC TGC AAC GTA GAT CAC Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His 195 200 205	684
10	AAG CCC AGC AAC ACC AAG GTG GAC AAG AGA GTT GGTGAGAGGC CAGCACAGGG Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val 210 215	737
	CAGGGAGGGT GTCTGCTGGA AGCCAGGCTC AGCCCTCCTG CCTGGACGCA CCCC GGCTGT	797
	GCAGCCCCAG CCCAGGGCAG CAAGGCATGC CCCATCTGTC TCCTCACCCG GAGGCCTCTG	857
	ACCACCCAC TCATGCTCAG GGAGAGGGTC TTCTGGATT TTCCACCAGG CTCCGGGCAG	917
15	CCACAGGCTG GATGCCCTA CCCCAGGCCC TCGCATACA GGGGCAGGTG CTGCGCTCAG	977
	ACCTGCCAAG AGCCATATCC GGGAGGACCC TGCCCTGAC CTAAGCCAC CCCAAAGGCC	1037
	AAACTCTCCA CTCCCTCAGC TCAGACACCT TCTCTCCTCC CAGATTCGAG TAACTCCCAA	1097
	TCTTCTCTCT GCA GAG TCC AAA TAT GGT CCC CCA TGC CCA TCA TGC CCA Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro 1 5 10	1146
20	GGTAAGCCAA CCCAGGCCTC GCCCTCCAGC TCAAGGCGGG ACAGGTGCCC TAGAGTAGCC	1206
	TGCATCCAGG GACAGGCCCC AGCCGGGTGC TGACGCATCC ACCTCCATCT CTTCTCAGC	1266
	A CCT GAG TTC CTG GGG GGA CCA TCA GTC TTC CTG TTC CCC CCA AAA Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys 1 5 10 15	1312
25	CCC AAG GAC ACT CTC ATG ATC TCC CGG ACC CCT GAG GTC ACG TGC GTG Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val 20 25 30	1360

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1	GTG GTG GAC GTG AGC CAG GAA GAC CCC GAG GTC CAG TTC AAC TGG TAC Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr	1408
	35 40 45	
	GTG GAT GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu	1456
	50 55 60	
5	CAG TTC AAC AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC ATG CAC Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Met His	1504
	65 70 75	
	CAG GAC TGG CTG AAC GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys	1552
	80 85 90 95	
10	GGC CTC CCG TCC TCC ATC GAG AAA ACC ATC TCC AAA GCC AAA Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys	1594
	100 105	
	GGTGGGACCC ACGGGGTGCG AGGGCCACAT GGACAGAGGT CAGCTCGGCC CACCCTCTGC	1654
	CCTGGGAGTG ACCGCTGTGC CAACCTCTGT CCCTACA GGG CAG CCC CGA GAG CCA Gly Gln Pro Arg Glu Pro	1709
	1 5	
15	CAG GTG TAC ACC CTG CCC CCA TCC CAG GAG GAG ATG ACC AAG AAC CAG Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln	1757
	10 15 20	
	GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAC CCC AGC GAC ATC GCC Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala	1805
	25 30 35	
20	GTG GAG TGG GAG AGT AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr	1853
	40 45 50	
	CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AGG CTA Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu	1901
	55 60 65 70	
25	ACC GTG GAC AAG AGC AGG TGG CAG GAG GGG AAT GTC TTC TCA GTC TCC Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Val Ser	1949
	75 80 85	

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1	GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACA CAG AAG AGC CTC TCC Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser 90 95 100	1997
	CTG TCT CTG GGT AAA TGAGTGCCAG GGCCGGCAAG CCCCCGCTCC CCGGGCTCTC Leu Ser Leu Gly Lys 105	2052
5	GGGGTCGCGC GAGGATGCTT GGCACGTACC CCGTCTACAT ACTTCCCAGG CACCCAGCAT GGAAATAAAG CACCCACCAC TGCCCTGGGC CCCTGTGAGA CTGTGATGGT TCTTTCCACG GGTCAGGCCG AGTCTGAGGC CTGAGTGACA TGAGGGAGGC AGAGCGGGTC CCACTGTCCC CACACTGGCC CAGGCTGTGC AGGTGTGCCT GGGCCACCTA GGGTGGGGCT CAGCCAGGGG CTGCCCTCGG CAGGGTGGGG GATTTGCCAG CGTGGCCCTC CCTCCAGCAG CAGGACTCTA	2112 2172 2232 2292 2352
10	GAGGATCATA ATCAGCCATA CCACATTTGT AGAGGTTTTA CTTGCTTTAA AAAACCTCCC ACACCTCCCC CTGAACCTGA AACATAAAAT GAATGCAATT GTTGTGTGTA ACTTGTTTAT TGCAGCTTAT AATGGTTACA AATAAAGCAA TAGCATCACA AATTTCACAA ATAAAGCATT TTTTTCACTG CATTCTAGTT GTGGTTTGTC CAAACTCATC AATGTATCTT ATCATGTCTG	2412 2472 2532 2592
15	GATCCTCTAC GCCGGACGCA TCGTGGCCGG CATCACCGGC GCCACAGGTG CGGTTGCTGG CGCCTATATC GCCGACATCA CCGATGGGGA AGATCGGGCT CGCCACTTCG GGCTCATGAG CGCTTGTTTC GCGTGGGTA TGGTGGCAGG CCCGTGGCCG GGGGACTGTT GGGCGCCATC TCCTTGCA TG CACCATTCCT TGCGGCGGCG GTGCTCAACG GCCTCAACCT ACTACTGGGC TGCTTCCTAA TGCAGGAGTC GCATAAGGGA GAGCGTCGAC CTCGGGCCGC GTTGCTGGCG	2652 2712 2772 2832 2892
20	TTTTTCCATA GGCTCCGCCC CCCTGACGAG CATCACAAAA ATCGACGCTC AAGTCAGAGG TGCGGAAACC CGACAGGACT ATAAAGATAC CAGGCGTTTC CCCCTGGAAG CTCCTCTGTG CGCTCTCCTG TTCCGACCCT GCCGCTTACC GGATACCTGT CCGCCTTTCT CCCTTCGGGA AGCGTGGCGC TTTCTCAATG CTCACGCTGT AGGTATCTCA GTTCGGTGTA GGTGCTTCGC	2952 3012 3072 3132
25	TCCAAGCTGG GCTGTGTGCA CGAACCCCCC GTTCAGCCCG ACCGCTGCGC CTTATCCGGT AACTATCGTC TTGAGTCCAA CCCGGTAAGA CACGACTTAT CGCCACTGGC AGCAGCCACT	3192 3252

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	GGTAACAGGA	TTAGCAGAGC	GAGGTATGTA	GGCGGTGCTA	CAGAGTTCTT	GAAGTGGTGG	3312
1	CCTAACTACG	GCTACACTAG	AAGGACAGTA	TTTGGTATCT	GCGCTCTGCT	GAAGCCAGTT	3372
	ACCTTCGGAA	AAAGAGTTGG	TAGCTCTTGA	TCCGGCAAAC	AAACCACCGC	TGGTAGCGGT	3432
	GGTTTTTTTG	TTTGCAAGCA	GCAGATTACG	CGCAGAAAAA	AAGGATCTCA	AGAAGATCCT	3492
	TTGATCTTTT	CTACGGGGTC	TGACGCTCAG	TGGAACGAAA	ACTCACGTTA	AGGGATTTTG	3552
5	GTCATGAGAT	TATCAAAAAG	GATCTTCACC	TAGATCCTTT	TAAATTAAAA	ATGAAGTTTT	3612
	AAATCAATCT	AAAGTATATA	TGAGTAAACT	TGGTCTGACA	GTTACCAATG	CTTAATCAGT	3672
	GAGGCACCTA	TCTCAGCGAT	CTGTCTATTT	CGTTCATCCA	TAGTTGCCTG	ACTCCCCGTC	3732
	GTGTAGATAA	CTACGATACG	GGAGGGCTTA	CCATCTGGCC	CCAGTGCTGC	AATGATACCG	3792
10	CGAGACCCAC	GCTCACCAGC	TCCAGATTTA	TCAGCAATAA	ACCAGCCAGC	CGGAAGGGCC	3852
	GAGCGCAGAA	GTGGTCCTGC	AACTTTATCC	GCCTCCATCC	AGTCTATTAA	TTGTTGCCGG	3912
	GAAGCTAGAG	TAAGTAGTTC	GCCAGTTAAT	AGTTTGCGCA	ACGTTGTTGC	CATTGCTACA	3972
	GGCATCGTGG	TGTCACGCTC	GTCGTTTGGT	ATGGCATCAT	TCAGCTCCGG	TTCCCAACGA	4032
	TCAAGGCGAG	TTACATGATC	CCCCATGTTG	TGCAAAAAAG	CGGTTAGCTC	CTTCGGTCCT	4092
15	CCGATCGTTG	TCAGAAGTAA	GTTGGCCGCA	GTGTTATCAC	TCATGGTTAT	GGCAGCACTG	4152
	CATAATTCTC	TTACTGTCAT	GCCATCCGTA	AGATGCTTTT	CTGTGACTGG	TGAGTACTCA	4212
	ACCAAGTCAT	TCTGAGAATA	GTGTATGCGG	CGACCGAGTT	GCTCTTGCCC	GGCGTCAACA	4272
	CGGGATAATA	CCGCGCCACA	TAGCAGAACT	TTAAAAGTGC	TCATCATTGG	AAAACGTTCT	4332
20	TCGGGGCGAA	AACTCTCAAG	GATCTTACCG	CTGTTGAGAT	CCAGTTCGAT	GTAACCCACT	4392
	CGTGCAACCA	ACTGATCTTC	AGCATCTTTT	ACTTTCACCA	GCGTTTCTGG	GTGAGCAAAA	4452
	ACAGGAAGGC	AAAATGCCGC	AAAAAAGGGA	ATAAGGGCGA	CACGGAAATG	TTGAATACTC	4512
	ATACTCTTCC	TTTTTCAATA	TTATTGAAGC	ATTATCAGG	GTTATTGTCT	CATGAGCGGA	4572
	TACATATTTG	AATGTATTTA	GAAAAATAAA	CAAATAGGGG	TTCCGCGCAC	ATTCCCCCGA	4632
25	AAAGTGCCAC	CTGACGTCTA	AGAAACCATT	ATTATCATGA	CATTAACCTA	TAAAAATAGG	4692

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CGTATCACGA GGCCCTGATG GCTCTTTGCG GCACCCATCG TTCGTAATGT TCCGTGGCAC 4752
1 CGACGACAAC CCTCAAGAGA AAATGTAATC AACTGGGCTC ACCTTCGGGT GGGCCTTTCT 4812
GCGTTTATAA GGAGACACTT TATGTTTAAG AAGGTTGGTA AATTCCTTGC GGCTTTGGCA 4872
GCCAAGCTAG AGATCTCTAG CTTCGTGTCA AGGACGGTGA CTGCAGTGAA TAATAAAATG 4932
TGTGTTTGTC CGAAATACGC GTTTTGAGAT TTCTGTGCGC GACTAAATTC ATGTCGCGCG 4992
5 ATAGTGGTGT TTATCGCCGA TAGAGATGGC GATATTGGAA AAATCGATAT TTGAAAATAT 5052
GGCATATTGA AAATGTCGCC GATGTGAGTT TCTGTGTAAC TGATATCGCC ATTTTTCCTAA 5112
AAGTGATTTT TGGGCATACG CGATATCTGG CGATAGCGCT TATATCGTTT ACGGGGGATG 5172
GCGATAGACG ACTTTGGTGA CTTGGGCGAT TCTGTGTGTC GCAAATATCG CAGTTTCGAT 5232
10 ATAGGTGACA GACGATATGA GGCTATATCG CCGATAGAGG CGACATCAAG CTGGCACATG 5292
GCCAATGCAT ATCGATCTAT ACATTGAATC AATATTGGCC ATTAGCCATA TTATTCATTG 5352
GTTATATAGC ATAAATCAAT ATTGGCTATT GGCCATTGCA TACGTTGTAT CCATATCATA 5412
ATATGTACAT TTATATTGGC TCATGTCCAA CATTACCGCC ATGTTGACAT TGATTATTGA 5472
CTAGTTATTA ATAGTAATCA ATTACGGGGT CATTAGTTCA TAGCCCATAT ATGGAGTTCC 5532
15 GCGTTACATA ACTTACGGTA AATGGCCCGC CTGGCTGACC GCCCAACGAC CCCC GCCCAT 5592
TGACGTCAAT AATGACGTAT GTTCCCATAG TAACGCCAAT AGGGACTTTC CATTGACGTC 5652
AATGGGTGGA GTATTTACGG TAAACTGCCC ACTTGGCAGT ACATCAAGTG TATCATATGC 5712
CAAGTACGCC CCCTATTGAC GTCAATGACG GTAAATGGCC CGCCTGGCAT TATGCCAGT 5772
20 ACATGACCTT ATGGGACTTT CCTACTTGGC AGTACATCTA CGTATTAGTC ATCGCTATTA 5832
CCATGGTGAT GCGGTTTTGG CAGTACATCA ATGGGCGTGG ATAGCGGTTT GACTCACGGG 5892
GATTTCCAAG TCTCCACCCC ATTGACGTCA ATGGGAGTTT GTTTTGGCAC CAAAATCAAC 5952
GGGACTTTCC AAAATGTCGT AACAACTCCG CCCCATTGAC GCAAATGGGC GGTAGGCGTG 6012
TACGGTGGGA GGTCTATATA AGCAGAGCTC GTTTAGTGAA CCGTCAGATC GCCTGGAGAC 6072
25 GCCATCCACG CTGTTTTGAC CTCCATAGAA GACACCGGGA CCGATCCAGC CTCCGCGGCC 6132

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	GGGAACGGTG CATTGGAACG CGGATTCCCC GTGCCAAGAG TGACGTAAGT ACCGCCTATA	6192
1	GAGTCTATAG GCCCAGCCCC TTGGCTTCTT ATGCATGCTA TACTGTTTTT GGCTTGGGGT	6252
	CTATACACCC CCGCTTCCTC ATGTTATAGG TGATGGTATA GCTTAGCCTA TAGGTGTGGG	6312
	TTATTGACCA TTATTGACCA CTCCCCTATT GGTGACGATA CTTTCCATTA CTAATCCATA	6372
	ACATGGCTCT TTGCCACAAC TCTCTTTATT GGCTATATGC CAATACACTG TCCTTCAGAG	6432
5	ACTGACACGG ACTCTGTATT TTTACAGGAT GGGGTCTCAT TTATTATTTA CAAATTCACA	6492
	TATACAACAC CACCGTCCCC AGTGCCCGCA GTTTTTATTA AACATAACGT GGGATCTCCA	6552
	CGCGAATCTC GGGTACGTGT TCCGGACATG GGCTCTTCTC CGGTAGCGGC GGAGCTTCTA	6612
	CATCCGAGCC CTGTCCCAT CCCTCCAGCG ACTCATGGTC GCTCGGCAGC TCCTTGCTCC	6672
10	TAACAGTGGA GGCCAGACTT AGGCACAGCA CGATGCCAC CACCACCAGT GTGCCGCACA	6732
	AGGCCGTGGC GGTAGGGTAT GTGTCTGAAA ATGAGCTCGG GGAGCGGGCT TGCACCGCTG	6792
	ACGCATTTGG AAGACTTAAG GCAGCGGCAG AAGAAGATGC AGGCAGCTGA GTTGTTGTGT	6852
	TCTGATAAGA GTCAGAGGTA ACTCCCGTTG CGGTGCTGTT AACGGTGGAG GGCAGTGTAG	6912
	TCTGAGCAGT ACTCGTTGCT GCCGCGCGCG CCACCAGACA TAATAGCTGA CAGACTAACA	6972
15	GACTGTTTCCT TTCCATGGGT CTTTCTGCA GTCACCGTCC TTGACACGAA GCTTGGGCTG	7032
	CAGGTCGATC GACTCTAGAG GATCGATCCC CGGGCGAGCT C	7073

(2) INFORMATION FOR SEQ ID NO:16:

- 20 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 219 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein

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-81-

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

1 Gly Val His Ser Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val
 1 5 10 15
 Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Phe Asn
 20 25 30
 Ile Lys Asp Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly
 5 35 40 45
 Leu Glu Trp Ile Gly Leu Ile Asp Pro Glu Asn Gly Asn Thr Ile Tyr
 50 55 60
 Asp Pro Lys Phe Gln Gly Arg Phe Ile Ile Ser Ala Asp Asn Ser Lys
 65 70 75 80
 Asn Thr Leu Phe Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala
 10 85 90 95
 Val Tyr Phe Cys Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr Trp Gly
 100 105 110
 Gln Gly Thr Pro Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
 115 120 125
 Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala
 15 130 135 140
 Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
 145 150 155 160
 Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
 165 170 175
 Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
 20 180 185 190
 Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His
 195 200 205
 Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val
 210 215

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-82-

(2) INFORMATION FOR SEQ ID NO:17:

- 1 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 12 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

- 5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro
 1 5 10

(2) INFORMATION FOR SEQ ID NO:18:

- 10 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 109 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

- 15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
 1 5 10 15

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
 20 25 30

20 Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val
 35 40 45

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
 50 55 60

Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Met His Gln
 65 70 75 80

25 Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly
 85 90 95

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1 Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys
100 105

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH: 107 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

10 Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu
1 5 10 15
Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe
20 25 30
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu
35 40 45
15 Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe
50 55 60
Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly
65 70 75 80
Asn Val Phe Ser Val Ser Val Met His Glu Ala Leu His Asn His Tyr
85 90 95
20 Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys
100 105

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH: 7864 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

1 (ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 9..711

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

5	AATTCACCAT GGGTGTGCCA ACTCAGGTAT TAGGATTACT GCTGCTGTGG CTTACAGATG	60
	CAAGATGTGA TATCCAAATG ACACAATCTC CTTCTTCTCT AAGTGCTTCT GTCGGAGATA	120
	GAGTAACAAT TACATGTAAG GCGAGTCAGG ACATTAGAAA GTATTTAAAC TGGTATCAGC	180
	AAAAACCTGG GAAGGCTCCT AAGCTACTGA TTTATTATGC AACAAGTTTG GCAGATGGAG	240
	TACCTTCTAG ATTTTCTGGT TCTGGCTCTG GAACAGACTA CACATTCACA ATTTCTTCTC	300
10	TCCAACCTGA GGACATTGCT ACATACTACT GCCTACAACA TGGTGAGAGT CCGTATACAT	360
	TTGGACAAGG AACAAACTA GAGATCACAA GAACTGTTGC GGCGCCGTCT GTCTTCATCT	420
	TCCCGCCATC TGATGAGCAG TTGAAATCTG GAACTGCCTC TGTGTGTGTC CTGCTGAATA	480
	ACTTCTATCC CAGAGAGGCC AAAGTACAGT GGAAGGTGGA TAACGCCCTC CAATCGGGTA	540
15	ACTCCCAGGA GAGTGTCA CA GAGCAGGACA GCAAGGACAG CACCTACAGC CTCAGCAGCA	600
	CCCTGACGCT GAGCAAAGCA GACTACGAGA AACACAAAGT CTACGCCTGC GAAGTCACCC	660
	ATCAGGGCCT GAGCTCGCCC GTCACAAAGA GCTTCAACAG GGGAGAGTGT TAGAGGGAGA	720
	AGTGCCCCCA CCTGCTCCTC AGTTCAGCC TGGGGATCAT AATCAGCCAT ACCACATTTG	780
	TAGAGGTTTT ACTTGCTTTA AAAAACCTCC CACACCTCCC CCTGAACCTG AAACATAAAA	840
20	TGAATGCAAT TGTGTGTGTT AACTTGTTTA TTGCAGCTTA TAATGGTTAC AAATAAAGCA	900
	ATAGCATCAC AAATTCACA AATAAAGCAT TTTTTCAC TGCATTCTAGT TGTGGTTTGT	960
	CCAAACTCAT CAATGTATCT TATCATGTCT GGATCCTCTA CGCCGGACGC ATCGTGGCCG	1020
	GCATCACCGG CGCCACAGGT GCGGTTGCTG GCGCCTATAT CGCCGACATC ACCGATGGGG	1080
25	AAGATCGGGC TCGCCACTTC GGGCTCATGA GCGCTTGTTT CGGCGTGGGT ATGGTGGCAG	1140

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1 GGTGCTCAAC GGCCTCAACC TACTACTGGG CTGCTTCCTA ATGCAGGAGT CGCATAAGGG 1260
AGAGCGTCGA CCTCGGGCCG CGTTGCTGGC GTTTTTCCAT AGGCTCCGCC CCCCTGACGA 1320
GCATCACAAA AATCGACGCT CAAGTCAGAG GTGGCGAAAC CCGACAGGAC TATAAAGATA 1380
CCAGGCGTTT CCCCTGGAA GCTCCCTCGT GCGCTCTCCT GTTCCGACCC TGCCGCTTAC 1440
5 CGGATACCTG TCCGCCTTTC TCCCTTCGGG AAGCGTGGCG CTTTCTCAAT GCTCACGCTG 1500
TAGGTATCTC AGTTCGGTGT AGGTCGTTCTG CTCCAAGCTG GGCTGTGTGC ACGAACCCCC 1560
CGTTCAGCCC GACCGCTGCG CCTTATCCGG TAACTATCGT CTTGAGTCCA ACCCGGTAAG 1620
ACACGACTTA TCGCCACTGG CAGCAGCCAC TGGTAACAGG ATTAGCAGAG CGAGGTATGT 1680
10 AGGCGGTGCT ACAGAGTTCT TGAAGTGGTG GCCTAACTAC GGCTACACTA GAAGGACAGT 1740
ATTTGGTATC TCGCTCTGCG TGAAGCCAGT TACCTTCGGA AAAAGAGTTG GTAGCTCTTG 1800
ATCCGGCAAA CAAACCACCG CTGGTAGCGG TGGTTTTTTT GTTTGCAAGC AGCAGATTAC 1860
GCGCAGAAAA AAAGGATCTC AAGAAGATCC TTTGATCTTT TCTACGGGGT CTGACGCTCA 1920
GTGGAACGAA AACTCACGTT AAGGGATTTT GGTCATGAGA TTATCAAAAA GGATCTTCAC 1980
15 CTAGATCCTT TTAAATTAAA AATGAAGTTT TAAATCAATC TAAAGTATAT ATGAGTAAAC 2040
TTGGTCTGAC AGTTACCAAT GCTTAATCAG TGAGGCACCT ATCTCAGCGA TCTGTCTATT 2100
TCGTTCATCC ATAGTTGCCT GACTCCCCGT CGTGTAGATA ACTACGATAC GGGAGGGCTT 2160
ACCATCTGGC CCCAGTGCTG CAATGATACC GCGAGACCCA CGCTCACC GGCTCAGATTT 2220
20 ATCAGCAATA AACCAGCCAG CCGGAAGGGC CGAGCGCAGA AGTGGTCCTG CAACTTTATC 2280
CGCCTCCATC CAGTCTATTA ATTGTTGCCG GGAAGCTAGA GTAAGTAGTT CGCCAGTTAA 2340
TAGTTTGCGC AACGTTGTTG CCATTGCTAC AGGCATCGTG GTGTCACGCT CGTCGTTTGG 2400
TATGGCTTCA TTCAGTCCG GTTCCCAACG ATCAAGGCGA GTTACATGAT CCCCCATGTT 2460
GTGCAAAAAA GCGGTTAGCT CCTTCGGTCC TCCGATCGTT GTCAGAAGTA AGTTGGCCGC 2520
25 AGTGTATCA CTCATGGTTA TGGCAGCACT GCATAATTCT CTTACTGTCA TGCCATCCGT 2580

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AAGATGCTTT TCTGTGACTG GTGAGTACTC AACCAAGTCA TTCTGAGAAT AGTGTATGCG 2640
1 GCGACCGAGT TGCTCTTGCC CGGCGTCAAC ACGGGATAAT ACCGCGCCAC ATAGCAGAAC 2700
TTTAAAAGTG CTCATCATTG GAAAACGTTC TTCGGGGCGA AAACCTCTCA GGATCTTACC 2760
GCTGTTGAGA TCCAGTTCGA TGTAACCCAC TCGTGACCCC AACTGATCTT CAGCATCTTT 2820
TACTTTTACC AGCGTTTCTG GGTGAGCAAA AACAGGAAGG CAAAATGCCG CAAAAAGGG 2880
5 AATAAGGGCG ACACGGAAAT GTTGAATACT CATACTCTTC CTTTTTCAAT ATTATTGAAG 2940
CATTTATCAG GGTTATTGTC TCATGAGCGG ATACATATTT GAATGTATTT AGAAAAATAA 3000
ACAAATAGGG GTTCCGCGCA CATTTCCCCG AAAAGTGCCA CCTGACGTCT AAGAAACCAT 3060
TATTATCATG ACATTAACCT ATAAAAATAG GCGTATCAG AGGCCCTGAT GGCTCTTTGC 3120
10 GGCACCCATC GTTCGTAATG TTCCGTGGCA CCGAGGACAA CCCTCAAGAG AAAATGTAAT 3180
CACACTGGCT CACCTTCGGG TGGGCCTTTC TCGCTTTATA AGGAGACACT TTATGTTTAA 3240
GAAGGTTGGT AAATTCCTTG CGGCTTTGGC AGCCAAGCTA GAGATCCGGC TGTGGAATGT 3300
GTGTCAGTTA GGGTGTGGAA AGTCCCCAGG CTCCCCAGCA GGCAGAAGTA TGCAAAGCAT 3360
GCATCTCAAT TAGTCAGCAA CCAGGCTCCC CAGCAGGCAG AAGTATGCAA AGCATGCATC 3420
15 TCAATTAGTC AGCAACCATA GTCCCCCCCC TAACTCCGCC CATCCCGCCC CTAACTCCGC 3480
CCAGTTCCGC CCATTCTCCG CCCCATGGCT GACTAATTTT TTTTATTTAT GCAGAGGCCG 3540
AGGCCGCCTC GGCCTCTGAG CTATTCCAGA AGTAGTGAGG AGGCTTTTTT GGAGGCCTAG 3600
GCTTTTGCAA AAAGCTAGCT TGGGGCCACC GCTCAGAGCA CCTTCCACCA TGGCCACCTC 3660
20 AGCAAGTTCC CACTTGAACA AAAACATCAA GCAAATGTAC TTGTGCCTGC CCCAGGGTGA 3720
GAAAGTCCAA GCCATGTATA TCTGGGTTGA TGGTACTGGA GAAGGACTGC GCTGCAAAAC 3780
CCGCACCCTG GACTGTGAGC CCAAGTGTGT AGAAGAGTTA CCTGAGTGGA ATTTTGATGG 3840
CTCTAGTACC TTTCAGTCTG AGGGCTCCAA CAGTGACATG TATCTCAGCC CTGTTGCCAT 3900
GTTTCGGGAC CCCTTCCGCA GAGATCCCAA CAAGCTGGTG TTCTGTGAAG TTTTCAAGTA 3960
25 CAACCGGAAG CCTGCAGAGA CCAATTTAAG GCACTCGTGT AAACGGATAA TGGACATGGT 4020

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GAGCAACCAG CACCCCTGGT TTGGAATGGA ACAGGAGTAT ACTCTGATGG GAACAGATGG 4080
1 GCACCCTTTT GGTGGCCTT CCAATGGCTT TCCTGGGCCC CAAGGTCCGT ATTACTGTGG 4140
TGTGGGCGCA GACAAAGCCT ATGGCAGGGA TATCGTGGAG GCTCACTACC GCGCCTGCTT 4200
GTATGCTGGG GTCAAGATTA CAGGAACAAA TGCTGAGGTC ATGCCTGCCC AGTGGGAACT 4260
CCAAATAGGA CCCTGTGAAG GAATCCGCAT GGGAGATCAT CTCTGGGTGG CCCGTTTCAT 4320
5 CTTNCATCGA GTATGTGAAG ACTTTGGGGT AATAGCAACC TTTGACCCCA AGCCCATTC 4380
TGGGAAGTGG AATGGTGCA GCTGCCATAC CAACTTTAGC ACCAAGGCCA TGCGGGAGGA 4440
GAATGGTCTG AAGCACATCG AGGAGGCCAT CGAGAAACTA AGCAAGCGGC ACCGGTACCA 4500
CATTGAGGCC TACGATCCCA AGGGGGGCCT GGACAATGCC CGTGGTCTGA CTGGGTTC 4560
10 CGAAACGTCC AACATCAACG ACTTTTCTGC TGGTGTGCGC AATCGCAGTG CCAGCATCCG 4620
CATTCCCCCG ACTGTGCGCC AGGAGAAGAA AGGTTACTTT GAAGACCGCG GCCCCTCTGC 4680
CAATTGTGAC CCCTTTGCAG TGACAGAAGC CATCGTCCGC ACATGCCTTC TCAATGAGAC 4740
TGGCCACGAG CCCTTCCAAT ACAAAAATA ATTAGACTTT GAGTGATCTT GAGCCTTTCC 4800
TAGTTCATCC CACCCCGCCC CAGAGAGATC TTTGTGAAG AACCTTACTT CTGTGGTGTG 4860
15 ACATAATTGG ACAAACTACC TACAGAGATT TAAAGCTCTA AGGTAAATAT AAAATTTTTA 4920
AGTGTATAAT GTGTTAACT ACTGATTCTA ATTGTTTGTG TATTTTAGAT TCCAACCTAT 4980
GGAAGTATG AATGGGAGCA GTGGTGAAT GCCTTTAATG AGGAAAACCT GTTTTGCTCA 5040
GAAGAAATGC CATCTAGTGA TGATGAGGCT ACTGCTGACT CTCAACATTC TACTCCTCCA 5100
20 AAAAGAAGA GAAAGGTAGA ACACCCAAG GACTTTCCTT CAGAATTGCT AAGTTTTTTG 5160
AGTCATGCTG TGTTTAGTAA TAGAACTCTT GCTTGCTTTG CTATTACAC CACAAAGGAA 5220
AAAGCTGCAC TGCTATACAA GAAAATTATG GAAAAATATT CTGTAACCTT TATAAGTAGG 5280
CATAACAGTT ATAATCATAA CATACTGTTT TTTCTTACTC CACACAGGCA TAGAGTGCT 5340
GCTATTAATA ACTATGCTCA AAAATTGTGT ACCTTTAGCT TTTAATTTG TAAAGGGGTT 5400
25 AATAAGGAAT ATTTGATGTA TAGTGCCTAG ACTAGAGATC ATAATCAGCC ATACCACATT 5460

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TGTAGAGGTT TTA CTTCCCTT TAAAAAACCT CCCACACCTC CCCCTGAACC TGAAACATAA 5520
1 AATGAATGCA ATTGTTGTTG TTA CTTGTT TATTGCAGCT TATAATGGTT ACAAATAAAG 5580
CAATAGCATC ACAAATTTCA CAAATAAAGC ATTTTTTTCA CTGCATTCTA GTTGTGGTTT 5640
GTCCAAACTC ATCAATGTAT CTTATCATGT CTGGATCTCT AGCTTCGTGT CAAGGACGGT 5700
GACTGCAGTG AATAATAAAA TGTGTGTTTG TCCGAAATAC GCGTTTTGAG ATTTCTGTCTG 5760
5 CCTACTAAAT TCATGTCGCG CGATAGTGGT GTTTATCGCC GATAGAGATG GCGATATTGG 5820
AAAAATCGAT ATTTGAAAAT ATGGCATATT GAAAATGTCG CCGATGTGAG TTTCTGTGTA 5880
ACTGATATCG CCATTTTTC AAAAGTGATT TTTGGGCATA CGCGATATCT GGCGATAGCG 5940
CTTATATCGT TTACGGGGGA TGGCGATAGA CGACTTTGGT GACTTGGGCG ATTCTGTGTG 6000
10 TCGCAAATAT CGCAGTTTCG ATATAGGTGA CAGACGATAT GAGGCTATAT CGCCGATAGA 6060
GGCGACATCA AGCTGGCACA TGGCCAATGC ATATCGATCT ATACATTGAA TCAATATTGG 6120
CCATTAGCCA TATTATTCAT TGGTTATATA GCATAAATCA ATATTGGCTA TTGGCCATTG 6180
CATACGTTGT ATCCATATCA TAATATGTAC ATTTATATTG GCTCATGTCC AACATTACCG 6240
CCATGTTGAC ATTGATTATT GACTAGTTAT TAATAGTAAT CAATTACGGG GTCATTAGTT 6300
15 CATAGCCCAT ATATGGAGTT CCGCGTTACA TAACTTACGG TAAATGGCCC GCCTGGCTGA 6360
CCGCCCCAAG ACCCCCGCCC ATTGACGTCA ATAATGACGT ATGTTCCCAT AGTAACGCCA 6420
ATAGGGACTT TCCATTGACG TCAATGGGTG GAGTATTTAC GGTAAACTGC CCACTTGGCA 6480
GTACATCAAG TGTATCATAT GCCAAGTACG CCCCTATTG ACGTCAATGA CGGTAAATGG 6540
20 CCCGCCTGGC ATTATGCCCA GTACATGACC TTATGGGACT TTCCTACTTG GCAGTACATC 6600
TACGTATTAG TCATCGCTAT TACCATGGTG ATGCGGTTTT GGCAGTACAT CAATGGGCGT 6660
GGATAGCGGT TTGACTCACG GGGATTTCCA AGTCTCCACC CCATTGACGT CAATGGGAGT 6720
TTGTTTTGGC ACCAAAATCA ACGGGACTTT CAAAATGTC GTAACAACTC CGCCCCATTG 6780
ACGCAAATGG GCGGTAGGCG TGTACGGTGG GAGGTCTATA TAAGCAGAGC TCGTTTAGTG 6840
25 AACCGTCAGA TCGCCTGGAG ACGCCATCCA CGCTGTTTTG ACCTCCATAG AAGACACCGG 6900

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GACCGATCCA GCCTCCGCGG CCGGGAACGG TGCATTGGAA CGCGGATTCC CCGTGCCAAG 6960
1 AGTGACGTAA GTACCGCCTA TAGAGTCTAT AGGCCACCCC CTTGGCTTC TTATGCATGC 7020
TATACTGTTT TTGGCTTCGG GTCTATACAC CCCCCTTCC TCATGTTATA GGTGATGGTA 7080
TAGCTTAGCC TATAGGTGTG GGTATTGAC CATTATTGAC CACTCCCCTA TTGGTGACGA 7140
TACTTTCCAT TACTAATCCA TAACATGGCT CTTTGCCACA ACTCTCTTTA TTGGCTATAT 7200
5 GCCAATACAC TGTCTTCAG AGACTGACAC GGACTCTGTA TTTTACAGG ATGGGGTCTC 7260
ATTTATTATT TACAAATTCA CATATACAAC ACCACCGTCC CCAGTGCCCG CAGTTTTTAT 7320
TAAACATAAC GTGGGATCTC CACGCGAATC TCGGGTACGT GTTCCGGACA TGGGCTCTTC 7380
TCCGGTAGCG GCGGAGCTTC TACATCCGAG CCCTGCTCCC ATGCCTCCAG CGACTCATGG 7440
10 TCGCTCGGCA TCTCCTTGCT CCTAACAGTG GAGGCCAGAC TTAGGCACAG CACGATGCCC 7500
ACCACCACCA GTGTGCCGCA CAAGGCCGTG GCGGTAGGGT ATGTGTCTGA AAATGAGCTC 7560
GGGGAGCGGG CTTGCACCGC TGACGCATTT GGAAGACTTA AGGCAGCGGC AGAAGAAGAT 7620
GCAGGCAGCT GAGTTGTTGT GTTCTGATAA GAGTCAGAGG TAACTCCCGT TCGGGTGCTG 7680
TTAACGGTGG AGGGCAGTGT AGTCTGAGCA GTACTCGTTG CTGCCGCGCG CGCCACCAGA 7740
15 CATAATAGCT GACAGACTAA CAGACTGTTC CTTTCCATGG GTCTTTTCTG CAGTCACCGT 7800
CCTTGACACG AAGCTTGGGC TGCAGGTCGA TCGACTCTAG AGGATCGATC CCCGGGCGAG 7860
CTCG 7864

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WHAT IS CLAIMED IS:

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1. A CDR-grafted antibody capable of inhibiting human tissue factor wherein the complementarity determining regions (CDRs) are derived from a non-human monoclonal antibody against tissue factor and the framework (FR) and constant (C) regions are derived from one or more human antibodies.

2. The CDR-grafted antibody of Claim 1 wherein said non-human monoclonal antibody is a murine antibody.

3. The CDR-grafted antibody of Claim 2 wherein said murine antibody is TF8-5G9.

4. The CDR-grafted antibody of Claim 1 wherein said CDRs of the heavy chain have the amino acid sequences:

CDR1	DDYMH	(SEQ ID NO:5)
CDR2	LIDPENGNTIYDPKFQG	(SEQ ID NO:6)
CDR3	DNSYYFDY	(SEQ ID NO:7)

and said CDRs of the light chain have the amino acid sequences:

CDR1	KASQDIRKYLN	(SEQ ID NO:8)
CDR2	YATSLAD	(SEQ ID NO:9)
CDR3	LQHGESPYT	(SEQ ID NO:10).

5. The CDR-grafted antibody of Claim 1 wherein the FR of the heavy chain is derived from the human antibody KOL.

6. The CDR-grafted antibody of Claim 1 wherein the FR of the light chain is derived from the human antibody REI.

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7. The CDR-grafted antibody of Claim 1
1 wherein the heavy chain variable region has the amino
acid sequence of SEQ ID NO:11.

8. The CDR-grafted antibody of Claim 1 or 7
wherein the light chain variable region has the amino
5 acid sequence of SEQ ID NO:12.

9. The CDR-grafted antibody of Claim 1
wherein the heavy chain variable region has the amino
acid sequence of SEQ ID NO:13.

10. The CDR-grafted antibody of Claim 1 or 9
10 wherein the light chain variable region has the amino
acid sequence of SEQ ID NO:14.

11. The CDR-grafted antibody of Claim 1
wherein the heavy chain constant region is the human
IgG4 constant region.

15 12. The CDR-grafted antibody of Claim 10
wherein the heavy chain constant region is the human
IgG4 constant region.

13. The CDR-grafted antibody of Claim 1
wherein the light chain constant region is the human
20 kappa constant region.

14. The CDR-grafted antibody of Claim 10
wherein the light chain constant region is the human
kappa constant region.

15. CDR-grafted monoclonal antibody TF8HCDR1
25 x TF8LCDR1.

16. CDR-grafted monoclonal antibody TF8HCDR20
x TF8LCDR3.

17. A fragment of the CDR-grafted antibody of
Claim 1 wherein said fragment is capable of inhibiting
30 human tissue factor.

18. The fragment of Claim 17 wherein said
1 fragment is an Fab or F(ab')₂ fragment.

19. A method of making the CDR-grafted
antibody of Claim 1 comprising cotransfecting a host
cell with an expression vector comprising a nucleic acid
5 encoding the CDR-grafted antibody heavy chain and an
expression vector comprising a nucleic acid encoding the
CDR-grafted antibody light chain; culturing the
transfected host cell; and recovering said CDR-grafted
antibody.

10 20. A method of making the CDR-grafted
antibody of Claim 1 comprising transfecting a host cell
with an expression vector comprising a nucleic acid
encoding the CDR-grafted antibody heavy chain and a
nucleic acid encoding the CDR-grafted antibody light
15 chain; culturing the transfected host cell; and
recovering said CDR-grafted antibody.

21. The method of Claim 18 or 19 wherein said
nucleic acid encoding the CDR-grafted antibody heavy
chain has the sequence of nucleotides 1-2360 of SEQ ID
20 NO:15.

22. The method of Claim 18 or 19 wherein said
nucleic acid encoding the CDR-grafted light chain has
the sequence of nucleotides 1-759 of SEQ ID NO:17.

23. The method of Claim 19 or 20 wherein said
25 host cell is a bacterial cell, yeast cell, insect cell
or mammalian cell.

24. The method of Claim 23 wherein said
mammalian cell is a CHO cell, COS cell or myeloma cell.

25. The method of Claim 19 wherein said
30 expression vector comprising a nucleic acid encoding the
CDR-grafted antibody heavy chain is pEe6TF8HCDR20.

26. The method of Claim 19 wherein said
1 expression vector comprising a nucleic acid encoding the
CDR-grafted antibody light chain is pEel2TF8LCDR3.

27. A nucleic acid encoding the heavy chain
of the CDR-grafted antibody of Claim 1.

5 28. A nucleic acid encoding the light chain
of the CDR-grafted antibody of Claim 1.

29. The nucleic acid of Claim 27 having the
sequence of nucleotides 1-2360 of SEQ ID NO:15.

30. The nucleic acid of Claim 28 having the
10 sequence of nucleotides 1-759 of SEQ ID NO:17.

31. A method of attenuation of coagulation
comprising administering a therapeutically effective
amount of a CDR-grafted antibody capable of inhibiting
human tissue factor to a patient in need of said
15 attenuation.

32. The method of Claim 31 wherein said CDR-
grafted antibody is TF8HCDR20 x TF84CDR3.

33. A method of treatment or prevention of
thrombotic disorder comprising administering a
20 therapeutically effective amount of a CDR-grafted
antibody capable of inhibiting human tissue factor to a
patient in need of said treatment or prevention.

34. The method of Claim 33 wherein said
thrombotic disorder is intravascular coagulation,
25 arterial restenosis or arteriosclerosis.

35. The method of Claim 33 or 34 wherein said
CDR-grafted antibody is TF8HCDR20 x TF8LCDR3.

36. A pharmaceutical composition comprising
at least one CDR-grafted antibody capable of inhibiting
30 human tissue factor and a pharmaceutically acceptable
carrier.

37. The pharmaceutical composition of Claim
1 36 wherein said CDR-grafted antibody is TF8HCDR20 x
TF8LCDR3.

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Sequence of the murine TF8-5G9 heavy chain cDNA with protein translation. The essential regions of the cDNA are as follows:

FIG. 1 A

<u>Nucleotides</u>	<u>Region</u>
1-10	5' untranslated region.
11-67	Start codon and leader sequence.
68-418	Variable region.
419-1390	Murine IgG1 constant region.
1391-1489	3' untranslated region.

Sequence Range: 1 to 1489

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      10      20      30      40
      *      *      *      *
GGT CCT TAC A ATG AAA TGC AGC TGG GTC ATC TTC TTC CTG ATG GCA GTG
CCA GGA ATG T TAC TTT ACG TCG ACC CAG TAG AAG AAG GAC TAC CGT CAC
      Met Lys Cys Ser Trp Val Ile Phe Phe Leu Met Ala Val>

50      60      70      80      90
  *      *      *      *      *
GTT ACA GGG GTC AAT TCA CAG ATT CAG CTG CAG CAG TCT GGG CCT GAG
CAA TGT CCC CAG TTA AGT CTC TAA GTC GAC GTC GTC AGA CCC CGA CTC
Val Thr Gly Val Asn Ser Glu Ile Gln Leu Gln Gln Ser Gly Ala Glu>

100     110     120     130     140
  *      *      *      *      *
CTT GTG AGG CCA GGG GCC TTA GTC AAG TTG TCC TGC AAA GCT TCT GGC
GAA CAC TCC GGT CCC CCG AAT CAG TTC AAC AGG ACG TTT CGA AGA CCG
Leu Val Arg Pro Gly Ala Leu Val Lys Leu Ser Cys Lys Ala Ser Gly>

150     160     170     180     190
  *      *      *      *      *
TTC AAC ATT AAA GAC TAC TAT ATG CAC TCG GTG AAG CAG AGG CCT GAA
AAG TTG TAA TTT CTG ATG ATA TAC GTG ACC CAC TTC GTC TCC GGA CTT
Phe Asn Ile Lys Asp Tyr Tyr Met His Trp Val Lys Gln Arg Pro Glu>

200     210     220     230     240
  *      *      *      *      *
CAG GGC CTG GAG TCG ATT CGA TTG ATT GAT CCT GAG AAT GGT AAT ACT
GTC CCG GAC CTC ACC TAA CCT AAC CTA GGA CTC TTA CCA TTA TGA
Gln Gly Leu Glu Trp Ile Gly Leu Ile Asp Pro Glu Asn Gly Asn Thr>

250     260     270     280
  *      *      *      *
ATA TAT GAC CCG AAG TTC CAG GGC AAG GCC AGT ATA ACA GCA GAC ACA
TAT ATA CTG GCC TTC AAG GTC CCG TTC CCG TCA TAT TGT CGT CTG TGT
Ile Tyr Asp Pro Lys Phe Gln Gly Lys Ala Ser Ile Thr Ala Asp Thr>

290     300     310     320     330
  *      *      *      *      *
TCC TCC AAC ACA GCC TAC CTG CAG CTC AGC AGC CTG ACA TCT GAG GAC
AGG AGG TTG TGT CCG ATG GAC GTC GAG TCG TCG GAC TGT AGA CTC CTG
Ser Ser Asn Thr Ala Tyr Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp>

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FIG. 1 B

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340      350      360      370      380
*      *      *      *      *
ACT GCC GTC TAT TAC TGT GCT AGA GAT AAC TCG TAC TAC TTT GAC TAC
TGA CGG CAG ATA ATG ACA CGA TCT CTA TTG AGC ATG ATG AAA CTG ATG
Thr Ala Val Tyr Tyr Cys Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr>

390      400      410      420      430
*      *      *      *      *
TGG GGC CAA GGC ACC ACT CTC ACA GTC TCC TCA GCC AAA ACG ACA CCC
ACC CCG GTT CCG TGG TGA GAG TGT CAG AGG AGT CCG TTT TGC TGT GGG
Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Pro>

440      450      460      470      480
*      *      *      *      *
CCA TCT GTC TAT CCA CTG GCC CCT GGA TCT GCT GCC CAA ACT AAC TCC
GGT AGA CAG ATA GGT GAC CGG GGA CCT AGA CGA CCG GTT TGA TTG AGG
Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser Ala Ala Gln Thr Asn Ser>

490      500      510      520
*      *      *      *
ATG GTG ACC CTG GGA TGC CTG GTC AAG GGC TAT TTC CCT GAG CCA GTG
TAC CAC TGG GAC CCT ACG GAC CAG TTC CCG ATA AAG GGA CTC GGT CAC
Met Val Thr Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val>

530      540      550      560      570
*      *      *      *      *
ACA GTG ACC TGG AAC TCT GGA TCC CTG TCC AGC GGT GTG CAC ACC TTC
TGT CAC TCG ACC TTG AGA CCT AGG GAC AGG TCG CCA CAC GTG TGG AAG
Thr Val Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe>

580      590      600      610      620
*      *      *      *      *
CCA GCT GTC CTG CAG TCT GAC CTC TAC ACT CTG AGC AGC TCA GTG ACT
GGT CGA CAG GAC GTC AGA CTG GAG ATG TGA GAC TCG TCG AGT CAC TGA
Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr>

630      640      650      660      670
*      *      *      *      *
GTG CCC TCC AGC ACC TGG CCC AGC GAG ACC GTC ACC TGC AAC GTT GCC
CAC GGG AGG TCG TCG ACC GCG TCG CTC TGG CAG TCG ACG TTG CAA CCG
Val Pro Ser Ser Thr Trp Pro Ser Glu Thr Val Thr Cys Asn Val Ala>

680      690      700      710      720
*      *      *      *      *
CAC CCG GCC AGC AGC ACC AAG GTG GAC AAG AAA ATT GTG CCC AGG GAT
GTG GGC CCG TCG TCG TGG TTC CAC CTG TTC TTT TAA CAC GGG TCC CTA
His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys Ile Val Pro Arg Asp>

730      740      750      760
*      *      *      *
TGT GGT TGT AAG CCT TGC ATA TGT ACA GTC CCA GAA GTA TCA TCT GTC
ACA CCA ACA TTC GGA ACC TAT ACA TGT CAG GGT CTT CAT AGT AGA CAG
Cys Gly Cys Lys Pro Cys Ile Cys Thr Val Pro Glu Val Ser Ser Val>

770      780      790      800      810
*      *      *      *      *
TTC ATC TTC CCC CCA AAG CCC AAG GAT GTG CTC ACC ATT ACT CTG ACT
AAG TAG AAG GCG GGT TTC GGG TTC CTA CAC GAG TGG TAA TGA GAC TGA
Phe Ile Phe Pro Pro Lys Pro Lys Asp Val Leu Thr Ile Thr Leu Thr>

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FIG. 1 C

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      820          830          840          850          860
      *           *           *           *           *
CCT AAG GTC ACG TGT GTT GTG GTA GAC ATC AGC AAG GAT GAT CCC GAG
GGA TTC CAG TGC ACA CAA CAC CAT CTG TAG TCG TTC CTA CTA GGG CTC
Pro Lys Val Thr Cys Val Val Val Asp Ile Ser Lys Asp Asp Pro Glu>

      870          880          890          900          910
      *           *           *           *           *
GTC CAG TTC AGC TGG TTT GTA GAT GAT GTG GAG GTG CAC ACA GCT CAG
CAG GTC AAG TCG ACC AAA CAT CTA CTA CAC CTC CAC GTG TGT CGA GTC
Val Gln Phe Ser Trp Phe Val Asp Asp Val Glu Val His Thr Ala Gln>

      920          930          940          950          960
      *           *           *           *           *
ACG CAA CCC CGG GAG GAG CAG TTC AAC AGC ACT TTC CGC TCA GTC AGT
TGC GTT GGG GCC CTC CTC GTC AAG TTG TCG TGA AAG GCG AGT CAG TCA
Thr Gln Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Ser Val Ser>

      970          980          990          1000
      *           *           *           *
GAA CTT CCC ATC ATG CAC CAG GAC TGG CTC AAT GGC AAG GAG TTC AAA
CTT GAA GCG TAG TAC GTG CTC CTG ACC GAG TTA CCG TTC CTC AAG TTT
Glu Leu Pro Ile Met His Gln Asp Trp Leu Asn Gly Lys Glu Phe Lys>

1010          1020          1030          1040          1050
      *           *           *           *           *
TGC AGG GTC AAC AGT GCA GCT TTC CCT GCC CCC ATC GAG AAA ACC ATC
ACG TCC CAG TTG TCA CGT CGA AAG GGA CCG GCG TAG CTC TTT TCG TAG
Cys Arg Val Asn Ser Ala Ala Phe Pro Ala Pro Ile Glu Lys Thr Ile>

1060          1070          1080          1090          1100
      *           *           *           *           *
TCC AAA ACC AAA GGC AGA CCG AAG GCT CCA CAG GTG TAC ACC ATT CCA
AGG TTT TCG TTT CCG TCT GGC TTC CGA CGT GTC CAC ATG TCG TAA GGT
Ser Lys Thr Lys Gly Arg Pro Lys Ala Pro Gln Val Tyr Thr Ile Pro>

1110          1120          1130          1140          1150
      *           *           *           *           *
CCT CCC AAG GAG CAG ATG GCC AAG GAT AAA GTC AGT CTC ACC TGC ATG
GGA GGG TTC CTC GTC TAC CCG TTC CTA TTT CAG TCA GAC TGG ACG TAC
Pro Pro Lys Glu Gln Met Ala Lys Asp Lys Val Ser Leu Thr Cys Met>

1160          1170          1180          1190          1200
      *           *           *           *           *
ATA ACA GAC TTC TTC CCT GAA GAC ATT ACT GTG GAG TCG CAG TCG AAT
TAT TGT CTG AAG AAG GGA CTT CTG TAA TGA CAC CTC ACC GTC ACC TTA
Ile Thr Asp Phe Phe Pro Glu Asp Ile Thr Val Glu Trp Gln Trp Asn>

1210          1220          1230          1240
      *           *           *           *
GGG CAG CCA GCG GAG AAC TAC AAG AAC ACT CAG CCC ATC ATG GAC ACA
CCC GTC GGT CGC CTC TTG ATG TTC TTG TGA GTC GGG TAG TAC CTG TGT
Gly Gln Pro Ala Glu Asn Tyr Lys Asn Thr Gln Pro Ile Met Asp Thr>

1250          1260          1270          1280          1290
      *           *           *           *           *
GAT GGC TCT TAC TTC GTC TAC AGC AAG CTC AAT GTG CAG AAG AGC AAC
CTA CCC AGA ATG AAG CAG ATG TCG TTC GAG TTA CAC GTC TTC TCG TTC
Asp Gly Ser Tyr Phe Val Tyr Ser Lys Leu Asn Val Gln Lys Ser Asn>

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FIG. 1 D

1300	1310	1320	1330	1340
TGG	GAG	GCA	GGA	AAT
ACT	TTC	ACC	TGC	TCT
GTG	TTA	CAT	GAG	GGC
CTG	ACC	CTC	CGT	CCT
TTA	TGA	AAG	TGG	ACG
AGA	CAC	AAT	GTA	CTC
CCG	GAC	Trp	Glu	Ala
Gly	Asn	Thr	Phe	Thr
Cys	Ser	Val	Leu	His
Glu	Gly	Leu		
1350	1360	1370	1380	1390
CAC	AAC	CAC	CAT	ACT
GAG	AAG	AGC	CTC	TCC
CAC	TCT	CCT	GGT	AAA
TG	ATC	GTG	TTG	GTG
GTA	TGA	CTC	TTC	TCG
GAG	AGG	GTG	AGA	GGA
CCA	TTT	AC	TAG	His
Asn	His	His	Thr	Glu
Lys	Ser	Leu	Ser	His
Ser	Pro	Gly	Lys	
1400	1410	1420	1430	1440
CCA	GTG	TCC	TTG	GAG
CCC	TCT	GGT	CCT	ACA
GGA	CTC	TGA	CAC	CTA
CCT	GGT	CAC	AGG	AAC
CTC	GGG	AGA	CCA	GGA
TGT	CCT	GAG	ACT	GTG
GAT	GGA			
1450	1460	1470	1480	
CCA	CCC	CTC	CCT	GTA
TAA	ATA	AAG	CAC	CCA
GCA	CTG	CCT	TGG	ACC
C	GGT	GGG	GAG	GGA
CAT	ATT	TAT	TTC	GTG
GGT	CGT	GAC	GGA	ACC
TGG	G			

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Sequence of the murine TF8-5G9 light chain cDNA with protein translation. The essential regions of the cDNA are as follows:

FIG. 2 A	<u>Nucleotides</u>	<u>Region</u>
	1-4	5' untranslated.
	5-64	Start codon and leader sequence.
	65-385	Variable region.
	386-706	Murine kappa constant region.
	707-917	3' untranslated region.
	918-937	Poly A tail.

Sequence Range: 1 to 937

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      10      20      30      40
      *      *      *      *
GGA C ATG CCG GCC CCT GCT CAG TTT TTT GGG ATC TTG TTG CTC TGG TTT
CCT G TAC GCC CCG GGA CGA GTC AAA AAA CCC TAG AAC AAC GAG ACC AAA
      Met Arg Ala Pro Ala Gln Phe Phe Gly Ile Leu Leu Leu Trp Phe>

50      60      70      80      90
      *      *      *      *
CCA GGT ATC AGA TGT CAC ATC AAG ATG ACC CAG TCT CCA TCC TCC ATG
GGT CCA TAG TCT ACA CTG TAG TTC TAC TGG GTC AGA GGT AGG AGG TAC
Pro Gly Ile Arg Cys Asp Ile Lys Met Thr Gln Ser Pro Ser Ser Met>

100     110     120     130     140
      *      *      *      *
TAT GCA TCG CTG GGA GAG AGA GTC ACT ATC ACT TGT AAG GCG AGT CAG
ATA CGT ACC GAC CCT CTC TCT CAG TGA TAG TGA ACA TTC GCG TCA GTC
Tyr Ala Ser Leu Gly Glu Arg Val Thr Ile Thr Cys Lys Ala Ser Gln>

150     160     170     180     190
      *      *      *      *
GAC ATT AGA AAG TAT TTA AAC TGG TAC CAG CAG AAA CCA TGG AAA TCT
CTG TAA TCT TTC ATA AAT TTG ACC ATG GTC GTC TTT GGT ACC TTT AGA
Asp Ile Arg Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Trp Lys Ser>

200     210     220     230     240
      *      *      *      *
CCT AAG ACC CTG ATC TAT TAT GCA ACA AGC TTG CCA GAT GCG GTC CCA
GGA TTC TCG GAC TAG ATA ATA CGT TGT TCG AAC CGT CTA CCC CAG GGT
Pro Lys Thr Leu Ile Tyr Tyr Ala Thr Ser Leu Ala Asp Gly Val Pro>

250     260     270     280
      *      *      *      *
TCA AGA TTC AGT GGC AGT CGA TCT GCG CAA GAT TAT TCT CTA ACC ATC
AGT TCT AAG TCA CCG TCA CCT AGA CCC GTT CTA ATA AGA GAT TCG TAG
Ser Arg Phe Ser Gly Ser Gly Ser Gly Gln Asp Tyr Ser Leu Thr Ile>

290     300     310     320     330
      *      *      *      *
AGC AGC CTG GAG TCT GAC GAT ACA GCA ACT TAT TAC TGT CTA CAA CAT
TCG TCG GAC CTC AGA CTG CTA TGT CGT TGA ATA ATG ACA GAT GTT GTA
Ser Ser Leu Glu Ser Asp Asp Thr Ala Thr Tyr Tyr Cys Leu Gln His>

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FIG. 2B

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340      350      360      370      380
*      *      *      *      *
GGT GAG AGC CCG TAC ACG TTC GGA GGG GGG ACC AAG CTG GAA ATA AAC
CCA CTC TCG GGC ATG TGC AAG CCT CCC CCC TGG TTC GAC CTT TAT TTG
Gly Glu Ser Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Asn>

390      400      410      420      430
*      *      *      *      *
AGG GCT GAT GCT GCA CCA ACT GTA TCC ATC TTC CCA CCA TCC AGT GAG
TCC CGA CTA CGA CGT GGT TGA CAT AGG TAG AAG GGT GGT AGG TCA CTC
Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu>

440      450      460      470      480
*      *      *      *      *
CAG TTA ACA TCT GGA GGT GCC TCA GTC GTG TGC TTC TTG AAC AAC TTC
GTC AAT TGT AGA CCT CCA CGG AGT CAG CAC ACG AAG AAC TTG TTG AAG
Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe>

490      500      510      520
*      *      *      *
TAC CCC AAA GAC ATC AAT GTC AAG TGG AAG ATT GAT GGC AGT GAA CGA
ATG GGG TTT CTG TAG TTA CAG TTC ACC TTC TAA CTA CCG TCA CTT GCT
Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg>

530      540      550      560      570
*      *      *      *      *
CAA AAT GGC GTC CTG AAC AGT TGG ACT GAT CAG GAC AGC AAA GAC AGC
GTT TTA CCG CAG GAC TTG TCA ACC TGA CTA GTC CTG TCG TTT CTG TCG
Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser>

580      590      600      610      620
*      *      *      *      *
ACC TAC AGC ATG AGC AGC ACC CTC ACG TTG ACC AAG GAC GAG TAT GAA
TGG ATG TCG TAC TCG TCG TGG GAG TGC AAC TGG TTC CTG CTC ATA CTT
Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu>

630      640      650      660      670
*      *      *      *      *
CGA CAT AAC AGC TAT ACC TGT GAG GCC ACT CAC AAG ACA TCA ACT TCA
GCT GTA TTG TCG ATA TGG ACA CTC CCG TGA GTG TTC TGT AGT TGA AGT
Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser>

680      690      700      710      720
*      *      *      *      *
CCC ATT GTC AAG AGC TTC AAC AGG AAT GAG TGT TA GAG ACA AAG GTC CTG
CGG TAA CAG TTC TCG AAG TTG TCC TTA CTC ACA AT CTC TGT TTC CAG GAC
Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys>

730      740      750      760      770
*      *      *      *      *
AGA CGC CAC CAC CAG CTC CCC AGC TCC ATC CTA TCT TCC CTT CTA AGG
TCT CGC GTG GTG GTC GAG CGG TCG AGG TAG GAT AGA AGG GAA GAT TCC

780      790      800      810
*      *      *      *
TCT TGG AGG CTT CCC CAC AAG CGA CCT ACC ACT GTT GCG GTG CTC CAA
AGA ACC TCC GAA GGG GTG TTC GCT GGA TCG TGA CAA CGC CAC GAG GTT

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FIG. 2 C

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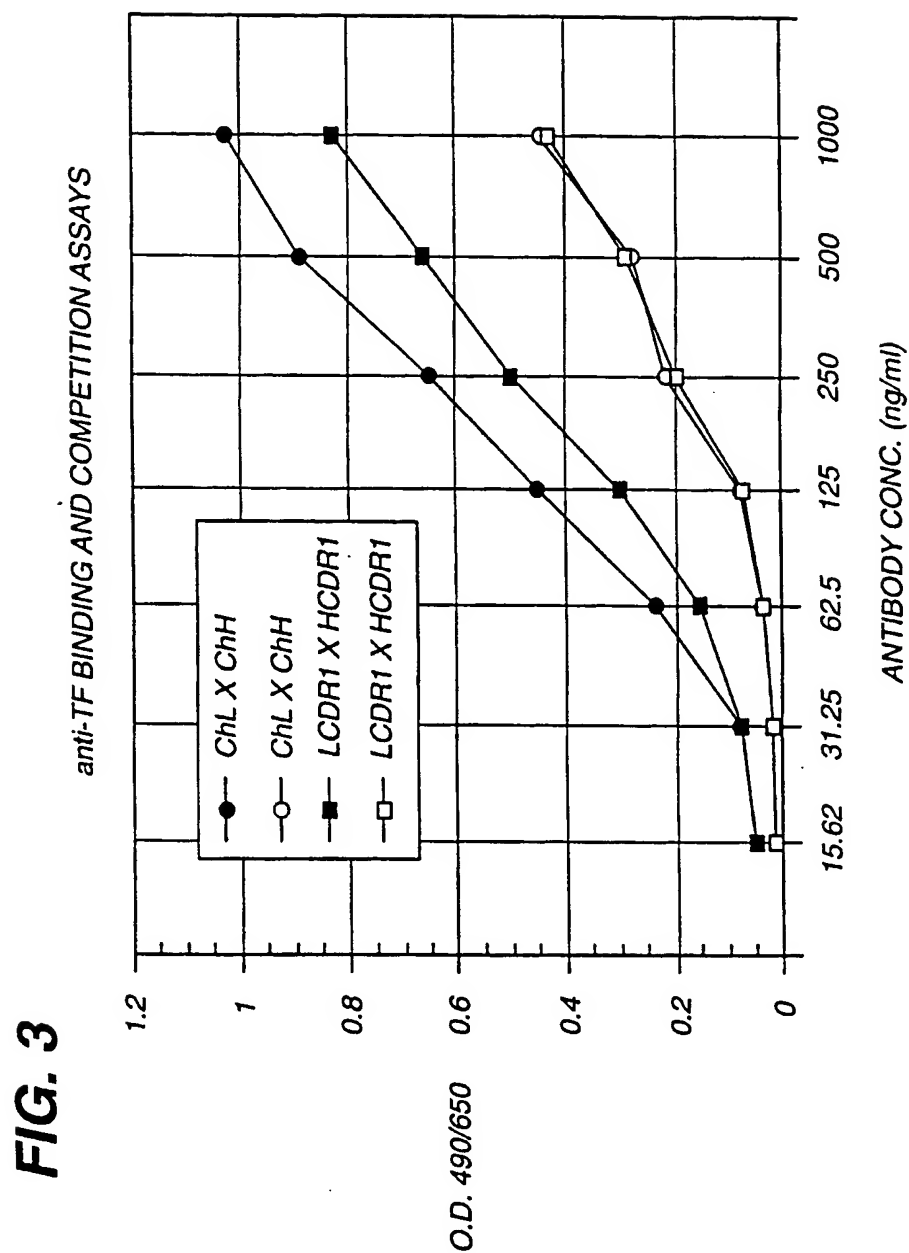
820      830      840      850      860
  *      *      *      *      *
ACC TCC TCC CCA CCT CCT TCT CCT CCT CCT CCC TTT CCT TGG CTT TTA
TGG AGG AGG GGT GGA GGA AGA GGA GGA GGA GGG AAA GGA ACC GAA AAT

870      880      890      900      910
  *      *      *      *      *
TCA TGC TAA TAT TTG CAG AAA ATA TTC AAT AAA GTG AGT CTT TGC ACT
AGT ACG ATT ATA AAC GTC TTT TAT AAG TTA TTT CAC TCA GAA ACG TGA

920      930
  *      *
TGA AAA AAA AAA AAA AAA AAA A
ACT TTT TTT TTT TTT TTT TTT T

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FIG. 4 A

The pEe6TF8HCDR20 expression vector DNA sequence. The coding regions of the TF8-5G9 CDR-grafted HC gene, TF8HCDR20, are translated.

Sequence Range: 1 to 7073

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      10      20      30      40
      *      *      *      *
GAA TTC GCC GCC ACC ATG GAA TGG AGC TGG GTC TTT CTC TTC TTC TTG
CTT AAG CGG CGG TGG TAC CTT ACC TCG ACC CAG AAA GAG AAG AAG AAC
      Met Glu Trp Ser Trp Val Phe Leu Phe Phe Leu>

50      60      70      80      90
      *      *      *      *
TCA GTA ACT ACA GGT GTA CAC TCA CAA GTT CAG CTG GTG GAG TCT GGA
AGT CAT TGA TGT CCA CAT GTG AGT GTT CAA GTC GAC CAC CTC AGA CCT
Ser Val Thr Thr Gly Val His Ser Gln Val Gln Leu Val Glu Ser Gly>

100     110     120     130     140
      *      *      *      *
GGA GGA GTA GTA CAA CCT GGA AGG TCA CTG AGA CTG TCT TGT AAG GCT
CCT CCT CAT CAT GTT GGA CCT TCC AGT GAC TCT GAC AGA ACA TTC CGA
Gly Gly Val Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala>

150     160     170     180     190
      *      *      *      *
AGT GGA TTC AAT ATC AAG GAC TAT TAT ATG CAC TGG GTC AGA CAA GCT
TCA CCT AAG TTA TAG TTC CTG ATA ATA TAC GTG ACC CAG TCT GTT CGA
Ser Gly Phe Asn Ile Lys Asp Tyr Tyr Met His Trp Val Arg Gln Ala>

200     210     220     230     240
      *      *      *      *
CCT GGA AAA GGA CTC GAG TGG ATA GGT TTA ATT GAT CCT GAG AAT GGT
GGA CCT TTT CCT GAG CTC ACC TAT CCA AAT TAA CTA GGA CTC TTA CCA
Pro Gly Lys Gly Leu Glu Trp Ile Gly Leu Ile Asp Pro Glu Asn Gly>

250     260     270     280
      *      *      *      *
AAC ACC ATA TAT GAT CCC AAG TTC CAA GGA AGA TTC ACA ATT TCT GCA
TTG TGC TAT ATA CTA GGG TTC AAG GTT CCT TCT AAG TGT TAA AGA CGT
Asn Thr Ile Tyr Asp Pro Lys Phe Gln Gly Arg Phe Thr Ile Ser Ala>

290     300     310     320     330
      *      *      *      *
GAC AAC TCT AAG AAT ACA CTG TTC CTG CAG ATG GAC TCA CTC AGA CCT
CTG TTG AGA TTC TTA TGT GAC AAG GAC GTC TAC CTG AGT GAG TCT GGA
Asp Asn Ser Lys Asn Thr Leu Phe Leu Gln Met Asp Ser Leu Arg Pro>

340     350     360     370     380
      *      *      *      *
GAG GAT ACA GCA GTC TAC TAT TGT GCT AGA GAT AAC AGT TAT TAC TTC
CTC CTA TGT CGT CAG ATG ATA ACA CCA TCT CTA TTG TCA ATA ATG AAG
Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Asp Asn Ser Tyr Tyr Phe>

390     400     410     420     430
      *      *      *      *
GAC TAC TGG GGC CAA GGA ACA CCA GTC ACC GTG AGC TCA GCT TCC ACC
CTG ATG ACC CCG GTT CCT TGT GGT CAG TGG CAC TCG AGT CGA AGG TGG
Asp Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser Ala Ser Thr>

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FIG. 4 B

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      440      450      460      470      480
      *      *      *      *      *
AAG GGC CCA TCC GTC TTC CCC CTG GCG CCC TGC TCC AGG AGC ACC TCC
TTC CCG GGT AGG CAG AAG GGG GAC CGC GGG ACG AGG TCC TCG TGG AGG
Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser>

      490      500      510      520
      *      *      *      *
GAG AGC ACA GCC GCC CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA
CTC TCG TGT CGG CGG GAC CCG ACG GAC CAG TTC CTG ATG AAG GGG CTT
Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu>

530      540      550      560      570
*      *      *      *      *
CCG GTG ACG GTG TCG TGG AAC TCA GGC GCC CTG ACC AGC GGC GTG CAC
GGC CAC TGC CAC AGC ACC TTG AGT CCG CGG GAC TGG TCG CCG CAC GTG
Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His>

      580      590      600      610      620
      *      *      *      *      *
ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA CTC TAC TCC CTC AGC AGC
TGG AAG GGC CGA CAG GAT GTC AGG AGT CCT GAG ATG AGG GAG TCG TCG
Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser>

      630      640      650      660      670
      *      *      *      *      *
GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACG AAG ACC TAC ACC TGC
CAC CAC TGG CAC GGG AGG TCG TCG AAC CCG TGC TTC TGG ATG TGG ACG
Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys>

      680      690      700      710      720
      *      *      *      *      *
AAC GTA GAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AGA GTT GGT
TTG CAT CTA GTG TTC GGG TCG TTG TGG TTC CAC CTG TTC TCT CAA CCA
Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val>

      730      740      750      760
      *      *      *      *
GAG AGG CCA CCA CAG GCC AGG GAG GGT GTC TGC TGG AAG CCA GGC TCA
CTC TCC GGT CGT GTC CCG TCC CTC CCA CAG ACG ACC TTC GGT CCG AGT

770      780      790      800      810
*      *      *      *      *
GCC CTC CTG CCT GGA CGC ACC CCG GCT GTG CAG CCC CAG CCC AGG GCA
CCG GAG GAC GGA CCT GCG TGG GGC CCA CAC GTC GGG GTC GGG TCC CGT

      820      830      840      850      860
      *      *      *      *      *
GCA AGG CAT GCC CCA TCT GTC TCC TCA CCC GGA GGC CTC TGA CCA CCC
CGT TCC GTA CCG GGT AGA CAG AGG AGT GGG CCT CCG GAG ACT GGT GGG

      870      880      890      900      910
      *      *      *      *      *
CAC TCA TGC TCA GGG AGA GGG TCT TCT GGA TTT TTC CAC CAG GCT CCG
GTG AGT ACG AGT CCC TCT CCC AGA AGA CCT AAA AAG GTG GTC CGA GGC

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FIG. 4 C

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      920      930      940      950      960
      *      *      *      *      *
GGC AGC CAC AGG CTG GAT GCC CCT ACC CCA GGC CCT GCG CAT ACA GGG
CCG TCG GTC TCC GAC CTA CCG GGA TGG GGT CCG GGA CGC GTA TGT CCC

      970      980      990      1000
      *      *      *      *
GCA GGT GCT GCG CTC AGA CCT GCC AAG AGC CAT ATC CCG GAG GAC CCT
CGT CCA CGA CGC GAG TCT GGA CCG TTC TCG GTA TAG GCC CTC CTG GGA

1010      1020      1030      1040      1050
      *      *      *      *      *
GCC CCT GAC CTA AGC CCA CCC CAA AGG CCA AAC TCT CCA CTC CCT CAG
CCG GGA CTG GAT TCG GGT GGG GTT TCC GGT TTG AGA GGT GAG GGA GTC

      1060      1070      1080      1090      1100
      *      *      *      *      *
CTC AGA CAC CTT CTC TCC TCC CAG ATT CGA GTA ACT CCC AAT CTT CTC
GAG TCT GTG GAA GAG AGG AGG GTC TAA GCT CAT TCA GGG TTA GAA GAG

      1110      1120      1130      1140      1150
      *      *      *      *      *
TCT GCA GAG TCC AAA TAT GGT CCC CCA TGC CCA TCA TGC CCA GGT AAG
AGA CGT CTC AGG TTT ATA CCA GGG GGT ACG GGT AGT ACG GGT CCA TTC
      Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro>

      1160      1170      1180      1190      1200
      *      *      *      *      *
CCA ACC CAG GCC TCG CCC TCC AGC TCA AGG CCG GAC AGG TGC CCT AGA
GGT TGG GTC CCG AGC GGG AGG TCG AGT TCC GCC CTC TCC ACG GGA TCT

      1210      1220      1230      1240
      *      *      *      *
GTA GCC TGC ATC CAG GGA CAG GCC CCA GCC GGG TGC TGA CGC ATC CAC
CAT CCG ACG TAG GTC CCT GTC CCG GGT CCG CCC ACG ACT GCG TAG GTG

1250      1260      1270      1280      1290
      *      *      *      *      *
CTC CAT CTC TTC CTC AGC A CCT GAG TTC CTG GGG GGA CCA TCA GTC TTC
GAG GTA GAG AAG GAG TCG T GGA CTC AAG GAC CCC CCT GGT AGT CAG AAG
      Pro Glu Phe Leu Gly Gly Pro Ser Val Phe>

1300      1310      1320      1330      1340
      *      *      *      *      *
CTG TTC CCC CCA AAA CCC AAG GAC ACT CTC ATG ATC TCC CCG ACC CCT
GAC AAG CCG GGT TTT CCG TTC CTG TGA GAG TAC TAG AGG GCC TCG GGA
Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro>

      1350      1360      1370      1380      1390
      *      *      *      *      *
GAG GTC ACG TGC GTC GTG GTG GAC GTC AGC CAG GAA GAC CCC GAG GTC
CTC CAG TGC ACG CAC CAC CAC CTG CAC TCG GTC CTT CTG GGG CTC CAG
Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val>

      1400      1410      1420      1430      1440
      *      *      *      *      *
CAG TTC AAC TCG TAC GTG GAT GGC GTG GAG GTG CAT AAT GCC AAG ACA
GTC AAG TTC ACC ATG CAC CTA CCG CAC CTC CAC GTA TTA CCG TTC TGT
Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr>

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FIG. 4 D

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      1450      1460      1470      1480
      *        *        *        *
AAG CCG CCG GAG GAG CAG TTC AAC AGC ACG TAC CGT GTG GTC AGC GTC
TTC GGC GCC CTC CTC GTC AAG TTG TCG TGC ATG GCA CAC CAG TCG CAG
Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val>

1490      1500      1510      1520      1530
*        *        *        *
CTC ACC GTC CTG CAC CAG GAC TGG CTG AAC GGC AAG GAG TAC AAG TGC
GAG TGG CAG GAC GTG GTC CTG ACC GAC TTG CCG TTC CTC ATG TTC ACG
Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys>

1540      1550      1560      1570      1580
*        *        *        *
AAG GTC TCC AAC AAA GGC CTC CCG TCC TCC ATC GAG AAA ACC ATC TCC
TTC CAG AGG TTG TTT CCG GAG GGC AGG AGG TAG CTC TTT TGG TAG AGG
Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser>

      1590      1600      1610      1620      1630
      *        *        *        *
AAA GCC AAA CG TCG GAC CCA CCG GGT GCG AGG CCC ACA TGG ACA GAG GTC
TTT CCG TTT CC ACC CTG GGT GCC CCA CCG TCC CCG TGT ACC TGT CTC CAG
Lys Ala Lys>

      1640      1650      1660      1670      1680
      *        *        *        *
AGC TCG GCC CAC CCT CTG CCC TGG GAG TGA CCG CTG TGC CAA CCT CTG
TCG AGC CCG GTG GGA GAC GGG ACC CTC ACT GGC GAC ACG GTT GGA GAC

      1690      1700      1710      1720      1730
      *        *        *        *
TCC CTA CA GGG CAG CCC CGA GAG CCA CAG GTG TAC ACC CTG CCC CCA TCC
AGG GAT GT CCC GTC GGG GGT CTC GGT GTC CAC ATG TGG GAC GGG GGT AGG
Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser>

      1740      1750      1760      1770      1780
      *        *        *        *
CAG GAG GAG ATG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA
GTC CTC CTC TAC TGG TTC TTG GTC CAG TCG GAC TGG ACG GAC CAG TTT
Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys>

      1790      1800      1810      1820
      *        *        *        *
GGC TTC TAC CCC AGC GAC ATC GCC GTG GAG TCG GAG AGC AAT GGC CAG
CCG AAG ATG GGG TCG CTG TAG CCG CAC CTC ACC CTC TCG TTA CCC GTC
Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln>

1830      1840      1850      1860      1870
*        *        *        *
CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC
GGC CTC TTC TTG ATC TTC TGG TGC GGA GGC CAC GAC CTG AGG CTC CCG
Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly>

1880      1890      1900      1910      1920
*        *        *        *
TCC TTC TTC CTC TAC AGC AGG CTA ACC GTG GAC AAG AGC AGG TGG CAG
ACG AAG AAG GAG ATG TCG TCC GAT TGG CAC CTG TTC TCG TCC ACC GTC
Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln>

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FIG. 4 E

1930	1940	1950	1960	1970
GAG GGG AAT GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTC CAC AAC				
CTC CCC TTA CAG AAG AGT ACG AGG CAC TAC GTA CTC CGA GAC GTG TTG				
Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn>				
1980	1990	2000	2010	2020
CAC TAC ACA CAG AAG AGC CTC TCC CTG TCT CTG GGT AAA T GAG TGC CAG				
GTG ATG TGT GTC TTC TCG GAG AGG GAC AGA GAC CCA TTT A CTC ACG GTC				
His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys Xxx>				
2030	2040	2050	2060	2070
GGC CGG CAA GCC CCC GCT CCC CGG GCT CTC GGG GTC GCG CGA GGA TGC				
CCG GCC GTT CGG GGG CGA GGG GCC CGA GAG CCC CAG CGC GCT CCT ACG				
2080	2090	2100	2110	
TTG GCA CGT ACC CCG TCT ACA TAC TTC CCA GGC ACC CAG CAT GGA AAT				
AAC CGT GCA TGG GGC AGA TGT ATG AAG GGT CCG TGG GTC GTA CCT TTA				
2120	2130	2140	2150	2160
AAA GCA CCC ACC ACT GCC CTG GGC CCC TGT GAG ACT GTG ATG GTT CTT				
TTT CGT GGG TGG TGA CCG GAC CCG GGG ACA CTC TGA CAC TAC CAA GAA				
2170	2180	2190	2200	2210
TCC ACG GGT CAG GCC GAG TCT GAG GCC TGA GTG ACA TGA GGG AGG CAG				
AGG TGC CCA GTC CCG CTC AGA CTC CCG ACT CAC TGT ACT CCC TCC GTC				
2220	2230	2240	2250	2260
AGC GGG TCC CAC TGT CCC CAC ACT GGC CCA GGC TGT GCA GGT GTG CCT				
TGG CCC AGG GTG ACA GGG GTG TGA CCG GGT CCG ACA CGT CCA CAC GGA				
2270	2280	2290	2300	2310
GGG CCA CCT AGG GTG GGG CTC AGC CAG GGG CTG CCC TCG GCA GGG TGG				
CCC GGT GGA TCC CAC CCC GAG TCG GTC CCC GAC GGG AGC CGT CCC ACC				
2320	2330	2340	2350	
GGG ATT TGC CAG CGT GGC CCT CCC TCC AGC AGC AGG ACT CTA GAG GAT				
CCC TAA ACC GTC GCA CCG GGA GGG AGG TCG TCG TCC TGA GAT CTC CTA				
2360	2370	2380	2390	2400
CAT AAT CAG CCA TAC CAC ATT TGT AGA GGT TTT ACT TGC TTT AAA AAA				
GTA TTA GTC GGT ATG GTC TAA ACA TCT CCA AAA TGA ACC AAA TTT TTT				
2410	2420	2430	2440	2450
CCT CCC ACA CCT CCC CCT GAA CCT GAA ACA TAA AAT GAA TGC AAT TGT				
CGA GGG TGT GGA GGG GGA CTT GGA CTT TGT ATT TTA CTT ACG TTA ACA				

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FIG. 4 F

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      2460      2470      2480      2490      2500
      *      *      *      *      *
TGT TGT TAA CTT GTT TAT TGC AGC TTA TAA TGG TTA CAA ATA AAG CAA
ACA ACA ATT GAA CAA ATA ACG TCG AAT ATT ACC AAT GTT TAT TTC GTT

      2510      2520      2530      2540      2550
      *      *      *      *      *
TAG CAT CAC AAA TTT CAC AAA TAA AGC ATT TTT TTC ACT GCA TTC TAG
ATC GTA GTG TTT AAA GTG TTT ATT TCG TAA AAA AAG TGA CGT AAG ATC

      2560      2570      2580      2590
      *      *      *      *
TTG TGG TTT GTC CAA ACT CAT CAA TGT ATC TTA TCA TGT CTG GAT CCT
AAC ACC AAA CAG GTT TGA GTA GTT ACA TAG AAT AGT ACA GAC CTA GGA

2600      2610      2620      2630      2640
*      *      *      *      *
CTA CGC CGG ACG CAT CGT GGC CGG CAT CAC CGG CGC CAC AGG TGC GGT
GAT GCG GCC TGC GTA GCA CCG GCC GTA GTG GCC GCG GTG TCC ACG CCA

      2650      2660      2670      2680      2690
      *      *      *      *      *
TGC TGG CGC CTA TAT CGC CGA CAT CAC CGA TGG GGA AGA TCG GGC TCG
ACG ACC GCG GAT ATA GCG GCT GTA GTG GCT ACC CCT TCT AGC CCG AGC

      2700      2710      2720      2730      2740
      *      *      *      *      *
CCA CTT CGG GCT CAT GAG CGC TTG TTT CGG CGT GGC TAT GGT GGC AGG
GGT GAA GCC CGA GTA CTC GCG AAC AAA GCC GCA CCC ATA CCA CCG TCC

      2750      2760      2770      2780      2790
      *      *      *      *      *
CCC GTG GCC GGG GGA CTG TTG GGC GCC ATC TCC TTG CAT GCA CCA TTC
GGG CAC CGG CCC CCT GAC AAC CCG CGG TAG AGG AAC GTA CGT GGT AAG

      2800      2810      2820      2830
      *      *      *      *
CTT CCG GCG GCG GTG CTC AAC GGC CTC AAC CTA CTA CTG GGC TGC TTC
GAA CCG CGC CGC CAC GAG TTG CCG GAG TTG GAT GAT GAC CCG ACG AAG

2840      2850      2860      2870      2880
*      *      *      *      *
CTA ATG CAG GAG TCG CAT AAG GGA GAG CGT CGA CCT CGG GCC GCG TTG
GAT TAC GTC CTC AGC GTA TTC CCT CTC GCA GCT GGA GCC CCG CGC AAC

      2890      2900      2910      2920      2930
      *      *      *      *      *
CTG GCG TTT TTC CAT AGG CTC CCG CCC CCT GAC GAG CAT CAC AAA AAT
GAC CCG AAA AAG GTA TCC GAG GCG GGG GGA CTG CTC GTA GTG TTT TTA

      2940      2950      2960      2970      2980
      *      *      *      *      *
CGA CCG TCA AGT CAG AGG TGG CGA AAC CCG ACA GGA CTA TAA AGA TAC
GCT GCG AGT TCA GTC TCC ACC GCT TTG GGC TGT CCT GAT ATT TCT ATG

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FIG. 4 G

```

      2990      3000      3010      3020      3030
      *      *      *      *      *
CAG GCG TTT CCC CCT GGA AGC TCC CTC GTG GCG TCT CCT GTT CCG ACC
GTC GCG AAA GGG GGA CCT TCG AGG GAG CAC GCG AGA GGA CAA GGC TGG

      3040      3050      3060      3070
      *      *      *      *
CTG CCG CTT ACC GGA TAC CTG TCC GCC TTT CTC CCT TCG GGA AGC GTG
GAC GGC GAA TGG CCT ATG GAC AGG CCG AAA GAG GGA AGC CCT TCG CAC

3080      3090      3100      3110      3120
      *      *      *      *      *
GCG CTT TCT CAA TGC TCA GCG TGT AGG TAT CTC AGT TCG GTG TAG GTC
CGC GAA AGA GTT ACG AGT GCG ACA TCC ATA GAG TCA AGC CAC ATC CAG

      3130      3140      3150      3160      3170
      *      *      *      *      *
GTT GCG TCC AAG CTG GGC TGT GTG CAC GAA CCC CCC GTT CAG CCC GAC
CAA GCG AGG TTC GAC CCG ACA CAC GTG CTT GGG GGG CAA GTC GCG CTC

      3180      3190      3200      3210      3220
      *      *      *      *      *
GCG TGC GCC TTA TCC GGT AAC TAT CGT CTT GAG TCC AAC CCG GTA AGA
GCG ACG CCG AAT AGG CCA TTG ATA GCA GAA CTC AGG TTG GGC CAT TCT

      3230      3240      3250      3260      3270
      *      *      *      *      *
CAC GAC TTA TCG CCA CTG GCA GCA GCC ACT GGT AAC AGG ATT AGC AGA
GTG CTG AAT AGC GGT GAC CGT CGT CCG TGA CCA TTG TCC TAA TCG TCT

      3280      3290      3300      3310
      *      *      *      *
GCG AGG TAT GTA GGC GGT GCT ACA GAG TTC TTG AAG TGG TGG CCT AAC
CGC TCC ATA CAT CCG CCA CGA TGT CTC AAG AAC TTC ACC ACC GGA TTG

3320      3330      3340      3350      3360
      *      *      *      *      *
TAC GGC TAC ACT AGA AGG ACA GTA TTT GGT ATC TGC GCT CTG CTG AAG
ATG CCG ATG TGA TCT TCC TGT CAT AAA CCA TAG ACG CGA GAC GAC TTC

      3370      3380      3390      3400      3410
      *      *      *      *      *
CCA GTT ACC TTC GGA AAA AGA GTT GGT AGC TCT TGA TCC GGC AAA CAA
GGT CAA TGG AAG CCT TTT TCT CAA CCA TCG AGA ACT AGG CCG TTT GTT

      3420      3430      3440      3450      3460
      *      *      *      *      *
ACC ACC GCT GGT AGC GGT GGT TTT TTT GTT TGC AAG CAG CAG ATT ACG
TGG TGG CGA CCA TCG CCA CCA AAA AAA CAA ACG TTC GTC GTC TAA TGC

      3470      3480      3490      3500      3510
      *      *      *      *      *
GCG AGA AAA AAA GGA TCT CAA GAA GAT CCT TTG ATC TTT TCT ACG GCG
GCG TCT TTT TTT CCT AGA GTT CTT CTA GGA AAC TAG AAA AGA TGC CCC

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FIG. 4 H

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      3520      3530      3540      3550
      *      *      *      *
TCT GAC GCT CAG TCG AAC GAA AAC TCA CGT TAA GGG ATT TTG GTC ATG
AGA CTC CGA GTC ACC TTG CTT TTG AGT GCA ATT CCC TAA AAC CAG TAC

3560      3570      3580      3590      3600
      *      *      *      *      *
AGA TTA TCA AAA AGG ATC TTC ACC TAG ATC CTT TTA AAT TAA AAA TGA
TCT AAT AGT TTT TCC TAG AAG TGG ATC TAG GAA AAT TTA ATT TTT ACT

      3610      3620      3630      3640      3650
      *      *      *      *      *
AGT TTT AAA TCA ATC TAA AGT ATA TAT GAG TAA ACT TGG TCT GAC AGT
TCA AAA TTT AGT TAG ATT TCA TAT ATA CTC ATT TGA ACC AGA CTG TCA

      3660      3670      3680      3690      3700
      *      *      *      *      *
TAC CAA TGC TTA ATC AGT GAG GCA CCT ATC TCA GCG ATC TGT CTA TTT
ATG GTT ACG AAT TAG TCA CTC CGT GGA TAG AGT CCG TAG ACA GAT AAA

      3710      3720      3730      3740      3750
      *      *      *      *      *
CGT TCA TCC ATA GTT GCC TGA CTC CCC GTC GTG TAG ATA ACT ACG ATA
GCA AGT AGG TAT CAA CGG ACT GAG GGG CAG CAC ATC TAT TGA TGC TAT

      3760      3770      3780      3790
      *      *      *      *
CGG GAG GGC TTA CCA TCT GGC CCC AGT GCT GCA ATG ATA CCG CGA GAC
GCC CTC CCG AAT GGT AGA CCG GGG TCA CGA CGT TAC TAT GCC GCT CTG

3800      3810      3820      3830      3840
      *      *      *      *      *
CCA CGC TCA CCG GCT CCA GAT TTA TCA GCA ATA AAC CAG CCA GCC GGA
GGT GCG AGT GGC CGA GGT CTA AAT AGT CGT TAT TTG GTC GGT CCG CCT

      3850      3860      3870      3880      3890
      *      *      *      *      *
AGG GCC GAG CGC AGA AGT GGT CCT GCA ACT TTA TCC GCC TCC ATC CAG
TCC CGG CTC GCG TCT TCA CCA GGA CGT TGA AAT AGG CCG AGG TAG GTC

      3900      3910      3920      3930      3940
      *      *      *      *      *
TCT ATT AAT TGT TGC CCG GAA GCT AGA GTA AGT AGT TCG CCA GTT AAT
AGA TAA TTA ACA ACG GCC CTT CGA TCT CAT TCA TCA AGC GGT CAA TTA

      3950      3960      3970      3980      3990
      *      *      *      *      *
AGT TTG CCG AAC GTT GTT GCC ATT GCT ACA GGC ATC GTG GTG TCA CCG
TCA AAC CCG TTG CAA CAA CCG TAA CGA TGT CCG TAG CAC CAC AGT GCC

      4000      4010      4020      4030
      *      *      *      *
TCG TCG TTT GGT ATG GCT TCA TTC AGC TCC GGT TCC CAA CGA TCA AGG
AGC AGC AAA CCA TAC CGA AGT AAG TCG AGG CCA AGG GTT GCT AGT TCC

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FIG. 4 I

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4040      4050      4060      4070      4080
      .           .           .           .           .
CGA GTT ACA TGA TCC CCC ATG TTG TGC AAA AAA GCG GTT AGC TCC TTC
GCT CAA TGT ACT AGG GGG TAC AAC ACG TTT TTT CGC CAA TCG AGG AAG

4090      4100      4110      4120      4130
      .           .           .           .           .
GGT CCT CCG ATC GTT GTC AGA AGT AAG TTG GCC GCA GTG TTA TCA CTC
CCA GGA GGC TAG CAA CAG TCT TCA TTC AAC CCG CGT CAC AAT AGT GAG

4140      4150      4160      4170      4180
      .           .           .           .           .
ATG GTT ATG GCA GCA CTG CAT AAT TCT CTT ACT GTC ATG CCA TCC GTA
TAC CAA TAC CGT CGT GAC GTA TTA AGA GAA TGA CAG TAC GGT AGG CAT

4190      4200      4210      4220      4230
      .           .           .           .           .
AGA TGC TTT TCT GTG ACT GGT GAG TAC TCA ACC AAG TCA TTC TGA GAA
TCT ACG AAA AGA CAC TGA CCA CTC ATG AGT TCG TTC AGT AAG ACT CTT

4240      4250      4260      4270
      .           .           .           .
TAG TGT ATG CCG CGA CCG AGT TGC TCT TGC CCG GCG TCA ACA CCG GAT
ATC ACA TAC GCC GCT GGC TCA ACG AGA ACG GGC CGC AGT TGT GCC CTA

4280      4290      4300      4310      4320
      .           .           .           .           .
AAT ACC GCG CCA CAT AGC AGA ACT TTA AAA GTG CTC ATC ATT GGA AAA
TTA TGG CGC GGT GTA TCG TCT TGA AAT TTT CAC GAG TAG TAA CCT TTT

4330      4340      4350      4360      4370
      .           .           .           .           .
CGT TCT TCG GGG CGA AAA CTC TCA AGG ATC TTA CCG CTG TTG AGA TCC
GCA AGA AGC CCC GCT TTT GAG AGT TCC TAG AAT GGC GAC AAC TCT AGG

4380      4390      4400      4410      4420
      .           .           .           .           .
AGT TCG ATG TAA CCC ACT CGT GCA CCC AAC TGA TCT TCA GCA TCT TTT
TCA AGC TAC ATT GGG TGA GCA CGT GGG TTG ACT AGA AGT CGT AGA AAA

4430      4440      4450      4460      4470
      .           .           .           .           .
ACT TTC ACC AGC GTT TCT GGG TGA GCA AAA ACA GGA AGG CAA AAT GCC
TGA AAG TCG TCG CAA AGA CCC ACT CGT TTT TGT CCT TCC GTT TTA CCG

4480      4490      4500      4510
      .           .           .           .
GCA AAA AAG GGA ATA AGC GCG ACA CCG AAA TGT TGA ATA CTC ATA CTC
CGT TTT TTC CCT TAT TCC CGC TGT GCC TTT ACA ACT TAT GAG TAT GAG

4520      4530      4540      4550      4560
      .           .           .           .           .
TTC CTT TTT CAA TAT TAT TGA AGC ATT TAT CAG GGT TAT TGT CTC ATG
AAG GAA AAA GTT ATA ATA ACT TCG TAA ATA GTC CCA ATA ACA GAG TAC

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FIG. 4 J

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4570      4580      4590      4600      4610
*         *         *         *         *
AGC CGA TAC ATA TTT GAA TGT ATT TAG AAA AAT AAA CAA ATA GCG GTT
TCG CCT ATG TAT AAA CTT ACA TAA ATC TTT TTA TTT GTT TAT CCC CAA

4620      4630      4640      4650      4660
*         *         *         *         *
CCG CGC ACA TTT CCC CGA AAA GTG CCA CCT GAC GTC TAA GAA ACC ATT
GGC CGC TGT AAA GGG GCT TTT CAC GGT CGA CTC CAG ATT CTT TGG TAA

4670      4680      4690      4700      4710
*         *         *         *         *
ATT ATC ATG ACA TTA ACC TAT AAA AAT AGG CGT ATC ACG AGG CCC TGA
TAA TAG TAC TGT AAT TCG ATA TTT TTA TCC CCA TAG TGC TCC GGG ACT

4720      4730      4740      4750
*         *         *         *
TGG CTC TTT GCG GCA CCC ATC GTT CGT AAT GTT CCG TCG CAC CGA GGA
ACC GAG AAA CGC CGT GGG TAG CAA GCA TTA CAA GGC ACC GTG GCT CCT

4760      4770      4780      4790      4800
*         *         *         *         *
CAA CCC TCA AGA GAA AAT GTA ATC ACA CTC GCT CAC CTT CCG GTG GGC
GTT GGG AGT TCT CTT TTA CAT TAG TGT GAC CGA GTG GAA GCC CAC CCG

4810      4820      4830      4840      4850
*         *         *         *         *
CTT TCT GCG TTT ATA AGG AGA CAC TTT ATG TTT AAG AAG GTT GGT AAA
GAA AGA CGC AAA TAT TCC TCT GTG AAA TAC AAA TTC TTC CAA CCA TTT

4860      4870      4880      4890      4900
*         *         *         *         *
TTC CTT GCG GCT TTG GCA GCC AAG CTA GAG ATC TCT AGC TTC GTG TCA
AAG GAA CGC CGA AAC CGT CGG TTC GAT CTC TAG AGA TCG AAG CAC AGT

4910      4920      4930      4940      4950
*         *         *         *         *
AGG ACG GTG ACT GCA GTG AAT AAT AAA ATG TGT GTT TGT CCG AAA TAC
TCC TGC CAC TGA CGT CAC TTA TTA TTT TAC ACA CAA ACA GGC TTT ATG

4960      4970      4980      4990
*         *         *         *
GCG TTT TGA GAT TTC TGT GCG CGA CTA AAT TCA TGT CCG GCG ATA GTG
CGC AAA ACT CTA AAG ACA GCG GCT GAT TTA AGT ACA GCG CGC TAT CAC

5000      5010      5020      5030      5040
*         *         *         *         *
GTG TTT ATC GCC GAT AGA GAT GCG GAT ATT GGA AAA ATC GAT ATT TGA
CAC AAA TAG CGG CTA TCT CTA CCG CTA TAA CCT TTT TAG CTA TAA ACT

5050      5060      5070      5080      5090
*         *         *         *         *
AAA TAT GGC ATA TTG AAA ATG TCG CCG ATG TGA GTT TCT GTG TAA CTG
TTT ATA CCG TAT AAC TTT TAC AGC GGC TAC ACT CAA AGA CAC ATT GAC

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FIG. 4 K

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      5100      5110      5120      5130      5140
      *        *        *        *        *
ATA TCG CCA TTT TTC CAA AAG TGA TTT TTG GGC ATA CGC GAT ATC TGG
TAT AGC GGT AAA AAG GTT TTC ACT AAA AAC CCG TAT GCG CTA TAG ACC

      5150      5160      5170      5180      5190
      *        *        *        *        *
CGA TAG CGC TTA TAT CGT TTA CCG GGG ATG GCG ATA GAC GAC TTT GGT
GCT ATC GCG AAT ATA GCA AAT GCC CCC TAC CCG TAT CTG CTG AAA CCA

      5200      5210      5220      5230
      *        *        *        *
GAC TTG GGC GAT TCT GTG TGT CCG AAA TAT CCG AGT TTC GAT ATA GGT
CTG AAC CCG CTA AGA CAC ACA GCG TTT ATA GCG TCA AAG CTA TAT CCA

5240      5250      5260      5270      5280
      *        *        *        *        *
GAC AGA CGA TAT GAG GCT ATA TCG CCG ATA GAG GCG ACA TCA AGC TGG
CTG TCT GCT ATA CTC CGA TAT AGC GGC TAT CTC CCG TGT AGT TCG ACC

      5290      5300      5310      5320      5330
      *        *        *        *        *
CAC ATG GCC AAT GCA TAT CGA TCT ATA CAT TGA ATC AAT ATT GGC CAT
GTG TAC CCG TTA CGT ATA GCT AGA TAT GTA ACT TAG TTA TAA CCG GTA

      5340      5350      5360      5370      5380
      *        *        *        *        *
TAG CCA TAT TAT TCA TTG GTT ATA TAG CAT AAA TCA ATA TTG GCT ATT
ATC GGT ATA ATA AGT AAC CAA TAT ATC GTA TTT AGT TAT AAC CGA TAA

      5390      5400      5410      5420      5430
      *        *        *        *        *
GGC CAT TGC ATA CGT TGT ATC CAT ATC ATA ATA TGT ACA TTT ATA TTG
CCG GTA ACG TAT GCA ACA TAG GTA TAG TAT TAT ACA TGT AAA TAT AAC

      5440      5450      5460      5470
      *        *        *        *
GCT CAT GTC CAA CAT TAC CCG CAT GTT GAC ATT GAT TAT TGA CTA GTT
CGA GTA CAG GTT GTA ATG GCG GTA CAA CTG TAA CTA ATA ACT GAT CAA

5480      5490      5500      5510      5520
      *        *        *        *        *
ATT AAT AGT AAT CAA TTA CCG GGT CAT TAG TTC ATA GCC CAT ATA TGG
TAA TTA TCA TTA GTT AAT GCC CCA GTA ATC AAG TAT CCG GTA TAT ACC

      5530      5540      5550      5560      5570
      *        *        *        *        *
AGT TCC GCG TTA CAT AAC TTA CCG TAA ATG GCC CCG CTG GCT GAC CCG
TCA AGG CCG AAT GTA TTG AAT GCC ATT TAC CCG GCG GAC CGA CTG GCG

      5580      5590      5600      5610      5620
      *        *        *        *        *
CCA ACG ACC CCC GCC CAT TGA CGT CAA TAA TGA CGT ATG TTC CCA TAG
GGT TGC TGG GCG CCG GTA ACT GCA GTT ATT ACT GCA TAC AAG GGT ATC

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FIG. 4 L

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      5630      5640      5650      5660      5670
      *      *      *      *      *
TAA CGC CAA TAG GGA CTT TCC ATT GAC GTC AAT GGG TGG AGT ATT TAC
ATT GCG GTT ATC CCT GAA AGG TAA CTG CAG TTA CCC ACC TCA TAA ATG

      5680      5690      5700      5710
      *      *      *      *
GGT AAA CTG CCC ACT TGG CAG TAC ATC AAG TGT ATC ATA TGC CAA GTA
CCA TTT GAC GGG TGA ACC GTC ATG TAG TTC ACA TAG TAT ACG GTT CAT

5720      5730      5740      5750      5760
*      *      *      *      *
CGC CCC CTA TTG ACG TCA ATG ACG GTA AAT GGC CCG CCT GGC ATT ATG
CGG GGG GAT AAC TGC AGT TAC TGC CAT TTA CCG GGC GGA CCG TAA TAC

      5770      5780      5790      5800      5810
      *      *      *      *      *
CCC AGT ACA TGA CCT TAT GGG ACT TTC CTA CTT GGC AGT ACA TCT ACG
GGG TCA TGT ACT GGA ATA CCC TGA AAG GAT GAA CCG TCA TGT AGA TGC

      5820      5830      5840      5850      5860
      *      *      *      *      *
TAT TAG TCA TCG CTA TTA CCA TGG TGA TGC GGT TTT GGC AGT ACA TCA
ATA ATC AGT AGC GAT AAT GGT ACC ACT ACG CCA AAA CCG TCA TGT AGT

      5870      5880      5890      5900      5910
      *      *      *      *      *
ATG GGC GTG GAT AGC GGT TTG ACT CAC GGG GAT TTC CAA GTC TCC ACC
TAC CCG CAC CTA TCG CCA AAC TGA GTG CCC CTA AAG GTT CAG AGG TGG

      5920      5930      5940      5950
      *      *      *      *
CCA TTG ACG TCA ATG GGA GTT TGT TTT GGC ACC AAA ATC AAC GGG ACT
GGT AAC TGC AGT TAC CCT CAA ACA AAA CCG TGG TTT TAG TTG CCC TGA

5960      5970      5980      5990      6000
*      *      *      *      *
TTC CAA AAT GTC GTA ACA ACT CCG CCC CAT TGA CGC AAA TGG GCG GTA
AAG GTT TTA CAG CAT TGT TGA GGC GGG GTA ACT GCG TTT ACC CCG CAT

      6010      6020      6030      6040      6050
      *      *      *      *      *
GGC GTG TAC GGT GGG AGG TCT ATA TAA GCA GAG CTC GTT TAG TGA ACC
CCG CAC ATG CCA CCC TCC AGA TAT ATT CGT CTC GAG CAA ATC ACT TGG

      6060      6070      6080      6090      6100
      *      *      *      *      *
GTC AGA TCG CCT GGA GAC GCC ATC CAC GCT GTT TTG ACC TCC ATA GAA
CAG TCT AGC GGA CCT CTG CCG TAG GTG CGA CAA AAC TGG AGG TAT CTT

      6110      6120      6130      6140      6150
      *      *      *      *      *
GAC ACC GGG ACC GAT CCA GCC TCC GCG GCC GGG AAC GGT GCA TTG GAA
CTG TGG CCC TGG CTA GGT CCG AGG CCG CCG CCC TTG CCA CGT AAC CTT

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FIG. 4 M

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        6160          6170          6180          6190
        *           *           *           *
CGC GGA TTC CCC GTG CCA AGA GTG ACG TAA GTA CCG CCT ATA GAG TCT
GCG CCT AAG GGG CAC GGT TCT CAC TGC ATT CAT GGC GGA TAT CTC AGA

6200          6210          6220          6230          6240
        *           *           *           *           *
ATA GGC CCA CCC CCT TGG CTT CTT ATG CAT GCT ATA CTG TTT TTG GCT
TAT CCG GGT GGG GGA ACC GAA GAA TAC GTA CGA TAT GAC AAA AAC CGA

        6250          6260          6270          6280          6290
        *           *           *           *           *
TGG GGT CTA TAC ACC CCC GCT TCC TCA TGT TAT AGG TGA TGG TAT AGC
ACC CCA GAT ATG TGG GGG CGA AGG AGT ACA ATA TCC ACT ACC ATA TCG

        6300          6310          6320          6330          6340
        *           *           *           *           *
TTA GCC TAT AGG TGT GGG TTA TTG ACC ATT ATT GAC CAC TCC CCT ATT
AAT CCG ATA TCC ACA CCC AAT AAC TGG TAA TAA CTG CTC AGG GGA TAA

        6350          6360          6370          6380          6390
        *           *           *           *           *
GGT GAC GAT ACT TTC CAT TAC TAA TCC ATA ACA TGG CTC TTT GCC ACA
CCA CTG CTA TGA AAG GTA ATG ATT AGG TAT TGT ACC GAG AAA CCG TGT

        6400          6410          6420          6430
        *           *           *           *
ACT CTC TTT ATT GGC TAT ATG CCA ATA CAC TGT CCT TCA GAG ACT GAC
TGA GAG AAA TAA CCG ATA TAC GGT TAT GTG ACA GGA AGT CTC TGA CTG

6440          6450          6460          6470          6480
        *           *           *           *           *
ACG GAC TCT GTA TTT TTA CAG GAT GGG GTC TCA TTT ATT ATT TAC AAA
TGC CTG AGA CAT AAA AAT GTC CTA CCC CAG AGT AAA TAA TAA ATG TTT

        6490          6500          6510          6520          6530
        *           *           *           *           *
TTC ACA TAT ACA ACA CCA CCG TCC CCA GTG CCC GCA GTT TTT ATT AAA
AAG TGT ATA TGT TGT GGT GGC AGG GGT CAC GGG CGT CAA AAA TAA TTT

        6540          6550          6560          6570          6580
        *           *           *           *           *
CAT AAC GTG GGA TCT CCA CGC GAA TCT CGG GTA CGT GTT CCG GAC ATG
GTA TTG CAC CCT AGA GGT GCG CTT AGA GCC CAT GCA CAA GGC CTG TAC

        6590          6600          6610          6620          6630
        *           *           *           *           *
GGC TCT TCT CCG GTA GCG GCG GAG CTT CTA CAT CCG AGC CCT GCT CCC
CCG AGA AGA GGC CAT CGC CGC CTC GAA GAT GTA GGC TCG GGA CGA GGG

        6640          6650          6660          6670
        *           *           *           *
ATG CCT CCA GCG ACT CAT GGT CGC TCG GCA GCT CCT TGC TCC TAA CAG
TAC GGA GGT CGC TGA GTA CCA GCG AGC CGT CGA GGA ACG AGG ATT GTC

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FIG. 4 N

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6680      6690      6700      6710      6720
  *      *      *      *      *
TGG AGG CCA GAC TTA GGC ACA GCA CGA TGC CCA CCA CCA CCA GTG TGC
ACC TCC GGT CTG AAT CCG TGT CGT GCT ACG GGT GGT GGT GGT CAC ACG

6730      6740      6750      6760      6770
  *      *      *      *      *
CGC ACA AGG CCG TGG CCG TAG GGT ATG TGT CTG AAA ATG AGC TCG GGG
GCG TGT TCC GGC ACC GCC ATC CCA TAC ACA GAC TTT TAC TCG AGC CCC

6780      6790      6800      6810      6820
  *      *      *      *      *
AGC GGG CTT GCA CCG CTG ACG CAT TTG GAA GAC TTA AGG CAG CCG CAG
TCG CCC GAA CGT GGC GAC TGC GTA AAC CTT CTG AAT TCC GTC GCC GTC

6830      6840      6850      6860      6870
  *      *      *      *      *
AAG AAG ATG CAG GCA GCT GAG TTG TTG TGT TCT GAT AAG AGT CAG ACG
TTC TTC TAC GTC CGT CGA CTC AAC AAC ACA AGA CTA TTC TCA GTC TCC

6880      6890      6900      6910
  *      *      *      *
TAA CTC CCG TTG CCG TGC TGT TAA CCG TGG AGG GCA GTG TAG TCT GAG
ATT GAG GGC AAC GCC ACG ACA ATT GCC ACC TCC CGT CAC ATC AGA CTC

6920      6930      6940      6950      6960
  *      *      *      *      *
CAG TAC TCG TTG CTG CCG CCG GCG CCA CCA GAC ATA ATA GCT GAC AGA
GTC ATG AGC AAC GAC GGC GCG CCG GGT GGT CTG TAT TAT CGA CTG TCT

6970      6980      6990      7000      7010
  *      *      *      *      *
CTA ACA GAC TGT TCC TTT CCA TGG GTC TTT TCT GCA GTC ACC GTC CTT
GAT TGT CTG ACA AGG AAA GGT ACC CAG AAA AGA CGT CAG TGG CAG GAA

7020      7030      7040      7050      7060
  *      *      *      *      *
GAC ACG AAG CTT GGC CTG CAG GTC GAT CGA CTC TAG AGG ATC GAT CCC
CTG TGC TTC GAA CCC GAC GTC CAG CTA GCT GAG ATC TCC TAG CTA GGG

7070
  *
CGG GCG AGC TC
GCC CCG TCG AG

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FIG. 5 A

The pEe12TF8LCDR3 expression vector DNA sequence. The coding regions of the TF8-5G9 CDR-grafted LC gene, TF8LCDR3, are translated.

Sequence Range: 1 to 7864

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      10      20      30      40      50
      *      *      *      *      *
AAT TCA CC ATG GGT GTG CCA ACT CAG GTA TTA GGA TTA CTG CTG CTG TCG
TTA AGT GG TAC CCA CAC GGT TGA GTC CAT AAT CCT AAT GAC GAC GAC ACC
      Met Gly Val Pro Thr Gln Val Leu Gly Leu Leu Leu Trp>

      60      70      80      90
      *      *      *      *
CTT ACA GAT GCA AGA TGT GAT ATC CAA ATG ACA CAA TCT CCT TCT TCT
GAA TGT CTA CGT TCT ACA CTA TAG GTT TAC TGT GTT AGA GGA AGA AGA
Leu Thr Asp Ala Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser>

100      110      120      130      140
      *      *      *      *      *
CTA AGT GCT TCT GTC GGA GAT AGA GTA ACA ATT ACA TGT AAG GCG AGT
GAT TCA CGA AGA CAG CCT CTA TCT CAT TGT TAA TGT ACA TTC CGC TCA
Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser>

      150      160      170      180      190
      *      *      *      *      *
CAG GAC ATT AGA AAG TAT TTA AAC TGG TAT CAG CAA AAA CCT GGG AAG
GTC CTG TAA TCT TTC ATA AAT TTC ACC ATA GTC GTT TTT GGA CCC TTC
Gln Asp Ile Arg Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys>

      200      210      220      230      240
      *      *      *      *      *
GCT CCT AAG CTA CTG ATT TAT TAT GCA ACA AGT TTC GCA GAT GGA GTA
CGA GGA TTC GAT GAC TAA ATA ATA CGT TGT TCA AAC CGT CTA CCT CAT
Ala Pro Lys Leu Leu Ile Tyr Tyr Ala Thr Ser Leu Ala Asp Gly Val>

      250      260      270      280      290
      *      *      *      *      *
CCT TCT AGA TTT TCT GGT TCT GGC TCT GGA ACA GAC TAC ACA TTC ACA
GGA AGA TCT AAA AGA CCA AGA CCC AGA CCT TGT CTG ATG TGT AAG TGT
Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr>

      300      310      320      330
      *      *      *      *
ATT TCT TCT CTC CAA CCT GAG GAC ATT GCT ACA TAC TAC TGC CTA CAA
TAA AGA AGA GAG GTT GGA CTC CTC TAA CGA TGT ATG ATG ACG GAT GTT
Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Leu Gln>

340      350      360      370      380
      *      *      *      *      *
CAT GGT GAG AGT CCG TAT ACA TTT GGA CAA GGA ACA AAA CTA GAG ATC
GTA CCA CTC TCA GGC ATA TGT AAA CCT GTT CCT TGT TTT GAT CTC TAG
His Gly Glu Ser Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile>

      390      400      410      420      430
      *      *      *      *      *
ACA AGA ACT GTT GCG GCG CCG TCT GTC TTC ATC TTC CCG CCA TCT GAT
TGT TCT TGA CAA CCG CCG GCG AGA CAG AAG TAG AAG GGC GGT AGA CTA
Thr Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp>

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FIG. 5 B

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      440      450      460      470      480
      *      *      *      *      *
GAG CAG TTG AAA TCT GGA ACT GCC TCT GTT GTG TGC CTG CTG AAT AAC
CTC GTC AAC TTT AGA CCT TGA CGG AGA CAA CAC ACG GAC GAC TTA TTG
Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn>

      490      500      510      520      530
      *      *      *      *      *
TTC TAT CCC AGA GAG GCC AAA GTA CAG TGG AAG GTG GAT AAC GCC CTC
AAG ATA GGG TCT CTC CGG TTT CAT GTC ACC TTC CAC CTA TTG CGG GAG
Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu>

      540      550      560      570
      *      *      *      *
CAA TCG GGT AAC TCC CAG GAG AGT GTC ACA GAG CAG GAC AGC AAG GAC
GTT AGC CCA TTG AGG GTC CTC TCA CAG TGT CTC GTC CTG TCG TTC CTG
Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp>

580      590      600      610      620
*      *      *      *      *
AGC ACC TAC AGC CTC AGC AGC ACC CTG ACG CTG AGC AAA GCA GAC TAC
TCG TGG ATG TCG GAG TCG TCG TGG GAC TGC GAC TCG TTT CGT CTG ATG
Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr>

      630      640      650      660      670
      *      *      *      *      *
GAG AAA CAC AAA GTC TAC GCC TGC GAA GTC ACC CAT CAG GGC CTG AGC
CTC TTT GTG TTT CAG ATG CGG ACG CTT CAG TGG GTA GTC CCG GAC TCG
Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser>

      680      690      700      710      720
      *      *      *      *      *
TCG CCC GTC ACA AAG AGC TTC AAC AGG GGA GAG TGT T AGA GGG AGA AGT
AGC GGG CAG TGT TTC TCG AAG TTG TCC CCT CTC ACA A TCT CCC TCT TCA
Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys>

      730      740      750      760      770
      *      *      *      *      *
GCC CCC ACC TGC TCC TCA GTT CCA GCC TGC GGA TCA TAA TCA GCC ATA
CGG GGG TGG ACG AGG AGT CAA GGT CGG ACC CCT AGT ATT AGT CGG TAT

      780      790      800      810
      *      *      *      *
CCA CAT TTG TAG AGG TTT TAC TTG CTT TAA AAA ACC TCC CAC ACC TCC
GGT GTA AAC ATC TCC AAA ATG AAC GAA ATT TTT TGG AGG GTG TGG AGG

820      830      840      850      860
*      *      *      *      *
CCC TGA ACC TGA AAC ATA AAA TGA ATG CAA TTG TTG TTG TTA ACT TGT
GGG ACT TGG ACT TTG TAT TTT ACT TAC GTT AAC AAC AAC AAT TGA ACA

      870      880      890      900      910
      *      *      *      *      *
TTA TTG CAG CTT ATA ATG GTT ACA AAT AAA GCA ATA GCA TCA CAA ATT
AAT AAC GTC GAA TAT TAC CAA TGT TTA TTT CGT TAT CGT AGT GTT TAA

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FIG. 5 C

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      920      930      940      950      960
      *      *      *      *      *
TCA CAA ATA AAG CAT TTT TTT CAC TGC ATT CTA GTT GTG GTT TGT CCA
AGT GTT TAT TTC GTA AAA AAA GTG ACG TAA GAT CAA CAC CAA ACA GGT

      970      980      990      1000      1010
      *      *      *      *      *
AAC TCA TCA ATG TAT CTT ATC ATG TCT GGA TCC TCT ACG CCG GAC GCA
TTG AGT AGT TAC ATA GAA TAG TAC AGA CCT AGG AGA TGC GGC CTG CGT

      1020      1030      1040      1050
      *      *      *      *
TCG TGG CCG GCA TCA CCG GCG CCA CAG GTG CCG TTG CTG GCG CCT ATA
AGC ACC GGC CGT AGT GGC CGC GGT GTC CAC GCC AAC GAC CGC GGA TAT

1060      1070      1080      1090      1100
*      *      *      *      *
TCG CCG ACA TCA CCG ATG GGG AAG ATC GGG CTC GCC ACT TCG GGC TCA
AGC GGC TGT AGT GGC TAC CCC TTC TAG CCC GAG CCG TGA AGC CCG AGT

1110      1120      1130      1140      1150
*      *      *      *      *
TGA GCG CTT GTT TCG GCG TGG GTA TGG TGG CAG GCC CGT GGC CCG GGG
ACT CGC GAA CAA AGC CGC ACC CAT ACC ACC GTC CCG GCA CCG GCC CCC

      1160      1170      1180      1190      1200
      *      *      *      *      *
ACT GTT GGG CCG CAT CTC CTT GCA TGC ACC ATT CCT TGC GGC GGC GGT
TGA CAA CCC GCG GTA GAG GAA CGT ACG TGG TAA GGA ACG CCG CCG CCA

      1210      1220      1230      1240      1250
      *      *      *      *      *
GCT CAA CCG CCT CAA CCT ACT ACT GGG CTG CTT CCT AAT GCA GGA GTC
CGA GTT GCC GGA GTT GGA TGA TGA CCC GAC GAA GGA TTA CGT CCT CAG

      1260      1270      1280      1290
      *      *      *      *
GCA TAA GGG AGA GCG TCG ACC TCG GGC CCG GTT GCT GGC GTT TTT CCA
CGT ATT CCC TCT CGC AGC TGG AGC CCG GCG CAA CGA CCG CAA AAA GGT

1300      1310      1320      1330      1340
*      *      *      *      *
TAG GCT CCG CCC CCC TGA CGA GCA TCA CAA AAA TCG ACG CTC AAG TCA
ATC CGA GGC GGG GGG ACT GCT CGT AGT GTT TTT AGC TGC GAG TTC AGT

1350      1360      1370      1380      1390
*      *      *      *      *
GAG GTG GCG AAA CCC GAC AGG ACT ATA AAG ATA CCA GGC GTT TCC CCC
CTC CAC CGC TTT GGG CTG TCC TGA TAT TTC TAT GGT CCG CAA AGG GGG

      1400      1410      1420      1430      1440
      *      *      *      *      *
TGG AAG CTC CCT CGT GCG CTC TCC TGT TCC GAC CCT GCC GCT TAC CCG
ACC TTC GAG GGA GCA CGC GAG AGG ACA AGG CTC GGA CCG CGA ATG GCC

      1450      1460      1470      1480      1490
      *      *      *      *      *
ATA CCT GTC CCG CTT TCT CCC TTC GGG AAG CGT GGC GCT TTC TCA ATG
TAT GGA CAG GCG GAA AGA GGG AAG CCC TTC GCA CCG CGA AAG AGT TAC

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FIG. 5 D

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      1500      1510      1520      1530
      *        *        *        *
CTC  ACC  CTG  TAG  GTA  TCT  CAG  TTC  GGT  GTA  GGT  CGT  TCG  CTC  CAA  GCT
GAG  TGC  GAC  ATC  CAT  AGA  GTC  AAG  CCA  CAT  CCA  GCA  AGC  GAG  GTT  CGA

1540      1550      1560      1570      1580
*        *        *        *        *
GGG  CTG  TGT  GCA  CGA  ACC  CCC  CGT  TCA  GCC  CGA  CCG  CTG  CGC  CTT  ATC
CCC  GAC  ACA  CGT  GCT  TGG  GGG  GCA  AGT  CGG  GCT  GGC  GAC  GCG  GAA  TAG

1590      1600      1610      1620      1630
*        *        *        *        *
CGG  TAA  CTA  TCG  TCT  TGA  GTC  CAA  CCC  GGT  AAG  ACA  CGA  CTT  ATC  GCC
GCC  ATT  GAT  AGC  AGA  ACT  CAG  GTT  GGG  CCA  TTC  TGT  GCT  GAA  TAG  CCG

      1640      1650      1660      1670      1680
      *        *        *        *        *
ACT  GGC  AGC  AGC  CAC  TGG  TAA  CAG  GAT  TAG  CAG  AGC  GAG  GTA  TGT  AGG
TGA  CCG  TCG  TCG  GTG  ACC  ATT  GTC  CTA  ATC  GTC  TCG  CTC  CAT  ACA  TCC

      1690      1700      1710      1720      1730
      *        *        *        *        *
CGG  TGC  TAC  AGA  GTT  CTT  GAA  GTG  GTG  GCC  TAA  CTA  CGG  CTA  CAC  TAG
GCC  ACG  ATG  TCT  CAA  GAA  CTT  CAC  CAC  CGG  ATT  GAT  GCC  GAT  GTG  ATC

      1740      1750      1760      1770
      *        *        *        *
AAG  GAC  AGT  ATT  TGG  TAT  CTG  CGC  TCT  GCT  GAA  GCC  AGT  TAC  CTT  CGG
TTC  CTG  TCA  TAA  ACC  ATA  GAC  GCG  AGA  CGA  CTT  CCG  TCA  ATG  GAA  GCC

1780      1790      1800      1810      1820
*        *        *        *        *
AAA  AAG  AGT  TGG  TAG  CTC  TTG  ATC  CGG  CAA  ACA  AAC  CAC  CGC  TGG  TAG
TTT  TTC  TCA  ACC  ATC  GAG  AAC  TAG  GCC  GTT  TGT  TTG  GTG  GCG  ACC  ATC

1830      1840      1850      1860      1870
*        *        *        *        *
CGG  TGG  TTT  TTT  TGT  TTG  CAA  GCA  GCA  GAT  TAC  GCG  CAG  AAA  AAA  AGG
GCC  ACC  AAA  AAA  ACA  AAC  GTT  CGT  CGT  CTA  ATG  CGC  GTC  TTT  TTT  TCC

      1880      1890      1900      1910      1920
      *        *        *        *        *
ATC  TCA  AGA  AGA  TCC  TTT  GAT  CTT  TTC  TAC  GGG  GTC  TGA  CGC  TCA  GTG
TAG  AGT  TCT  TCT  AGG  AAA  CTA  GAA  AAG  ATG  CCC  CAG  ACT  GCG  AGT  CAC

      1930      1940      1950      1960      1970
      *        *        *        *        *
GAA  CGA  AAA  CTC  ACC  TTA  AGG  GAT  TTT  GGT  CAT  GAG  ATT  ATC  AAA  AAG
CTT  GCT  TTT  GAG  TGC  AAT  TCC  CTA  AAA  CCA  GTA  CTC  TAA  TAG  TTT  TTC

      1980      1990      2000      2010
      *        *        *        *
GAT  CTT  CAC  CTA  GAT  CCT  TTT  AAA  TTA  AAA  ATG  AAG  TTT  TAA  ATC  AAT
CTA  GAA  GTG  GAT  CTA  CGA  AAA  TTT  AAT  TTT  TAC  TTC  AAA  ATT  TAG  TTA

2020      2030      2040      2050      2060
*        *        *        *        *
CTA  AAG  TAT  ATA  TGA  GTA  AAC  TTG  GTC  TGA  CAG  TTA  CCA  ATG  CTT  AAT
GAT  TTC  ATA  TAT  ACT  CAT  TTG  AAC  CAG  ACT  GTC  AAT  GGT  TAC  GAA  TTA

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FIG. 5 E

2070	2080	2090	2100	2110
CAG TGA GGC ACC TAT CTC AGC GAT CTG TCT ATT TCG TTC ATC CAT AGT				
GTC ACT CCG TGG ATA GAG TCG CTA GAC AGA TAA AGC AAG TAG GTA TCA				
2120	2130	2140	2150	2160
TGC CTG ACT CCC CGT CGT GTA GAT AAC TAC GAT ACG GGA GGG CTT ACC				
ACG GAC TGA GGG GCA GCA CAT CTA TTG ATG CTA TGC CCT CCC GAA TGG				
2170	2180	2190	2200	2210
ATC TGG CCC CAG TGC TGC AAT GAT ACC GCG AGA CCC ACG CTC ACC GGC				
TAG ACC GGG GTC ACG ACG TTA CTA TGG CCG TCT GGG TGC GAG TGG CCG				
2220	2230	2240	2250	
TCC AGA TTT ATC AGC AAT AAA CCA GCC AGC CCG AAG GGC CGA GCG CAG				
AGG TCT AAA TAG TCG TTA TTT GGT CCG TCG GCC TTC CCG GCT CGC GTC				
2260	2270	2280	2290	2300
AAG TGG TCC TGC AAC TTT ATC CCG CTC CAT CCA GTC TAT TAA TTG TTG				
TTC ACC AGG ACG TTG AAA TAG CCG GAG GTA GGT CAG ATA ATT AAC AAC				
2310	2320	2330	2340	2350
CCG GGA AGC TAG AGT AAG TAG TTC GCC AGT TAA TAG TTT GCG CAA CGT				
GGC CCT TCG ATC TCA TTC ATC AAG CCG TCA ATT ATC AAA CCG GTT GCA				
2360	2370	2380	2390	2400
TGT TGC CAT TGC TAC AGG CAT CGT GGT GTC ACG CTC GTC GTT TGG TAT				
ACA ACG GTA ACG ATG TCC GTA GCA CCA CAG TGC GAG CAG CAA ACC ATA				
2410	2420	2430	2440	2450
GGC TTC ATT CAG CTC CCG TTC CCA ACG ATC AAG GCG AGT TAC ATG ATC				
CCG AAG TAA GTC GAG GCC AAG GGT TGC TAG TTC CCG TCA ATG TAC TAG				
2460	2470	2480	2490	
CCC CAT GTT GTG CAA AAA AGC GGT TAG CTC CTT CCG TCC TCC GAT CGT				
GGG GTA CAA CAC GTT TTT TCG CCA ATC GAG GAA GCC AGG AGG CTA GCA				
2500	2510	2520	2530	2540
TGT CAG AAG TAA GTT GGC CCG AGT GTT ATC ACT CAT GGT TAT GGC AGC				
ACA GTC TTC ATT CAA CCG GCG TCA CAA TAG TGA GTA CCA ATA CCG TCG				
2550	2560	2570	2580	2590
ACT GCA TAA TTC TCT TAC TGT CAT GCC ATC CGT AAG ATG CTT TTC TGT				
TGA CGT ATT AAG AGA ATG ACA GTA CCG TAG GCA TTC TAC GAA AAG ACA				
2600	2610	2620	2630	2640
GAC TGG TGA GTA CTC AAC CAA GTC ATT CTG AGA ATA GTG TAT GCG GCG				
CTG ACC ACT CAT GAG TTG GTT CAG TAA GAC TCT TAT CAC ATA CCG CCG				

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FIG. 5 F

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      2650      2660      2670      2680      2690
      *         *         *         *         *
ACC GAG TTG CTC TTG CCC GGC GTC AAC ACG GGA TAA TAC CGC GCC ACA
TGG CTC AAC GAG AAC GGG CCG CAG TTG TGC CCT ATT ATG GCG CCG TGT

      2700      2710      2720      2730
      *         *         *         *
TAG CAG AAC TTT AAA AGT GCT CAT CAT TGG AAA ACG TTC TTC GGG GCG
ATC GTC TTG AAA TTT TCA CGA GTA GTA ACC TTT TGC AAG AAG CCC CCG

2740      2750      2760      2770      2780
*         *         *         *         *
AAA ACT CTC AAG GAT CTT ACC GCT GTT GAG ATC CAG TTC GAT GTA ACC
TTT TGA GAG TTC CTA GAA TGG CGA CAA CTC TAG GTC AAG CTA CAT TGG

2790      2800      2810      2820      2830
*         *         *         *         *
CAC TCG TGC ACC CAA CTG ATC TTC AGC ATC TTT TAC TTT CAC CAG CGT
GTG AGC ACC TGG GTT GAC TAG AAG TCG TAG AAA ATG AAA GTG GTC GCA

      2840      2850      2860      2870      2880
      *         *         *         *         *
TTC TGG GTG AGC AAA AAC AGG AAG GCA AAA TGC CGC AAA AAA GGG AAT
AAG ACC CAC TCG TTT TTG TCC TTC CGT TTT ACG GCG TTT TTT CCC TTA

      2890      2900      2910      2920      2930
      *         *         *         *         *
AAG GGC GAC ACG GAA ATG TTG AAT ACT CAT ACT CTT CCT TTT TCA ATA
TTC CCG CTG TGC CTT TAC AAC TTA TGA GTA TGA GAA GGA AAA AGT TAT

      2940      2950      2960      2970
      *         *         *         *
TTA TTG AAG CAT TTA TCA GGG TTA TTG TCT CAT GAG CGG ATA CAT ATT
AAT AAC TTC GTA AAT AGT CCC AAT AAC AGA GTA CTC GCC TAT GTA TAA

2980      2990      3000      3010      3020
*         *         *         *         *
TGA ATG TAT TTA GAA AAA TAA ACA AAT AGG GGT TCC GCG CAC ATT TCC
ACT TAC ATA AAT CTT TTT ATT TGT TTA TCC CCA AGG CCG GTG TAA AGG

3030      3040      3050      3060      3070
*         *         *         *         *
CCG AAA AGT GCC ACC TGA CGT CTA AGA AAC CAT TAT TAT CAT GAC ATT
GGC TTT TCA CCG TGG ACT GCA GAT TCT TTG GTA ATA ATA GTA CTG TAA

      3080      3090      3100      3110      3120
      *         *         *         *         *
AAC CTA TAA AAA TAG GCG TAT CAC GAG GCC CTG ATG GCT CTT TGC GGC
TTG GAT ATT TTT ATC CCG ATA GTG CTC CCG GAC TAC CGA GAA ACG CCG

      3130      3140      3150      3160      3170
      *         *         *         *         *
ACC CAT CGT TCG TAA TGT TCC GTG GCA CCG AGG ACA ACC CTC AAG AGA
TGG GTA GCA AGC ATT ACA AGG CAC CGT GGC TCC TGT TGG GAG TTC TCT

      3180      3190      3200      3210
      *         *         *         *
AAA TGT AAT CAC ACT GGC TCA CCT TCG GGT GGG CCT TTC TGC GTT TAT
TTT ACA TTA GTG TGA CCG AGT GGA AGC CCA CCC GGA AAG ACG CAA ATA

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FIG. 5 G

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3220      3230      3240      3250      3260
*          *          *          *          *
AAG GAG ACA CTT TAT GTT TAA GAA GGT TGG TAA ATT CCT TGC GGC TTT
TTC CTC TGT GAA ATA CAA ATT CTT CCA ACC ATT TAA GGA ACG CCG AAA

3270      3280      3290      3300      3310
*          *          *          *          *
GGC AGC CAA GCT AGA GAT CCG GCT GTG GAA TGT GTG TCA GTT AGG GTG
CCG TCG GTT CGA TCT CTA GGC CGA CAC CTT ACA CAC AGT CAA TCC CAC

3320      3330      3340      3350      3360
*          *          *          *          *
TGG AAA GTC CCC AGG CTC CCC AGC AGG CAG AAG TAT GCA AAG CAT GCA
ACC TTT CAG GGG TCC GAG GGG TCG TCC GTC TTC ATA CGT TTC GTA CGT

3370      3380      3390      3400      3410
*          *          *          *          *
TCT CAA TTA GTC AGC AAC CAG GCT CCC CAG CAG GCA GAA GTA TGC AAA
AGA GTT AAT CAG TCG TTG GTC CGA GGG GTC GTC CGT CTT CAT ACG TTT

3420      3430      3440      3450
*          *          *          *
GCA TGC ATC TCA ATT AGT CAG CAA CCA TAG TCC CGC CCC TAA CTC CGC
CGT ACG TAG AGT TAA TCA GTC GTT GGT ATC AGG GCG GGG ATT GAG GCG

3460      3470      3480      3490      3500
*          *          *          *          *
CCA TCC CGC CCC TAA CTC CGC CCA GTT CCG CCC ATT CTC CGC CCC ATG
GGT AGG GCG GGG ATT GAG GCG GGT CAA GGC GGG TAA GAG GCG GCG TAC

3510      3520      3530      3540      3550
*          *          *          *          *
GCT GAC TAA TTT TTT TTA TTT ATG CAG AGG CCG AGG CCG CCT CGG CCT
CGA CTG ATT AAA AAA AAT AAA TAC GTC TCC GGC TCC GGC GGA GCC GGA

3560      3570      3580      3590      3600
*          *          *          *          *
CTG AGC TAT TCC AGA AGT AGT GAG GAG GCT TTT TTG GAG GCC TAG GCT
GAC TCG ATA AGG TCT TCA TCA CTC CTC CGA AAA AAC CTC CGG ATC CGA

3610      3620      3630      3640      3650
*          *          *          *          *
TTT GCA AAA AGC TAG CTT GGG GCC ACC GCT CAG AGC ACC TTC CAC CAT
AAA CGT TTT TCG ATC GAA CCC CGG TGG CGA GTC TCG TGG AAG GTG GTA

3660      3670      3680      3690
*          *          *          *
GGC CAC CTC AGC AAG TTC CCA CTT GAA CAA AAA CAT CAA GCA AAT GTA
CCG GTG GAG TCG TTC AAG CGT GAA CTT GTT TTT GTA GTT CGT TTA CAT

3700      3710      3720      3730      3740
*          *          *          *          *
CTT GTC CCT GCC CCA GGG TGA GAA AGT CCA AGC CAT GTA TAT CTG GGT
GAA CAC GGA CCG GGT CCC ACT CTT TCA GGT TCG GTA CAT ATA GAC CCA

3750      3760      3770      3780      3790
*          *          *          *          *
TGA TGG TAC TGG AGA AGG ACT GCG CTG CAA AAC CCG CAC CCT GGA CTG
ACT ACC ATG ACC TCT TCC TGA CCG GAC GTT TTG GGC GTG GGA CCT GAC

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FIG. 5 H

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      3800      3810      3820      3830      3840
      *        *        *        *        *
TGA GCC CAA GTG TGT AGA AGA GTT ACC TGA GTG GAA TTT TGA TGG CTC
ACT CGG GTT CAC ACA TCT TCT CAA TGG ACT CAC CTT AAA ACT ACC GAG

      3850      3860      3870      3880      3890
      *        *        *        *        *
TAG TAC CTT TCA GTC TGA GGG CTC CAA CAG TGA CAT GTA TCT CAG CCC
ATC ATG GAA AGT CAG ACT CCC GAG GTT GTC ACT GTA CAT AGA GTC GGG

      3900      3910      3920      3930
      *        *        *        *
TGT TGC CAT GTT TCG GGA CCC CTT CCG CAG AGA TCC CAA CAA GCT GGT
ACA ACG GTA CAA AGC CCT GGG GAA GGC GTC TCT AGG GTT GTT CGA CCA

3940      3950      3960      3970      3980
*        *        *        *        *
GTT CTG TGA AGT TTT CAA GTA CAA CCG GAA GCC TGC AGA GAC CAA TTT
CAA GAC ACT TCA AAA GTT CAT GTT GGC CTT CCG ACG TCT CTG GTT AAA

3990      4000      4010      4020      4030
*        *        *        *        *
AAG GCA CTC GTG TAA ACG GAT AAT GGA CAT GGT GAG CAA CCA GCA CCC
TTC CGT GAG CAC ATT TGC CTA TTA CCT GTA CCA CTC GTT GGT CGT GGG

      4040      4050      4060      4070      4080
      *        *        *        *        *
CTG GTT TGG AAT GGA ACA GGA GTA TAC TCT GAT GGG AAC AGA TGG GCA
GAC CAA ACC TTA CCT TGT CCT CAT ATG AGA CTA CCC TTG TCT ACC CGT

      4090      4100      4110      4120      4130
      *        *        *        *        *
CCC TTT TGG TTG GCC TTC CAA TGG CTT TCC TGG GCC CCA AGG TCC GTA
GGG AAA ACC AAC CCG AAG GTT ACC GAA AGG ACC CCG GGT TCC AGG CAT

      4140      4150      4160      4170
      *        *        *        *
TTA CTG TGG TGT GGG CGC AGA CAA AGC CTA TGG CAG GGA TAT CGT GGA
AAT GAC ACC ACA CCC GCG TCT GTT TCG GAT ACC GTC CCT ATA GCA CCT

4180      4190      4200      4210      4220
*        *        *        *        *
GGC TCA CTA CCG CGC CTG CTT GTA TGC TGG GGT CAA GAT TAC AGG AAC
CCG AGT GAT GGC GCG GAC GAA CAT ACG ACC CCA GTT CTA ATG TCC TTG

4230      4240      4250      4260      4270
*        *        *        *        *
AAA TGC TGA GGT CAT GCC TGC CCA GTG GGA ACT CCA AAT AGG ACC CTG
TTT ACG ACT CCA GTA CCG ACG GGT CAC CCT TGA GGT TTA TCC TGG GAC

      4280      4290      4300      4310      4320
      *        *        *        *        *
TGA AGG AAT CCG CAT GCG AGA TCA TCT CTG GGT GGC CCG TTT CAT CTT
ACT TCC TTA GGC GTA CCC TCT AGT AGA GAC CCA CCG GGC AAA GTA GAA

      4330      4340      4350      4360      4370
      *        *        *        *        *
NCA TCG AGT ATG TGA AGA CTT TGG GGT AAT AGC AAC CTT TGA CCC CAA
NGT AGC TCA TAC ACT TCT GAA ACC CCA TTA TCG TTG GAA ACT GGG GTT

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FIG. 5 I

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      4380      4390      4400      4410
      *        *        *        *
GCC CAT TCC TGG GAA CTG GAA TGG TGC AGG CTG CCA TAC CAA CTT TAG
CGG GTA AGG ACC CTT GAC CTT ACC ACG TCC GAC GGT ATG GTT GAA ATC

4420      4430      4440      4450      4460
      *        *        *        *        *
CAC CAA GGC CAT GCG GGA GGA GAA TGG TCT GAA GCA CAT CGA GGA GGC
GTG GTT CCG GTA CCG CCT CCT CTT ACC AGA CTT CGT GTA GCT CCT CCG

4470      4480      4490      4500      4510
      *        *        *        *        *
CAT CGA GAA ACT AAG CAA GCG GCA CCG GTA CCA CAT TCG AGC CTA CGA
GTA GCT CTT TGA TTC GTT CCG CGT GGC CAT GGT GTA AGC TCG GAT GCT

      4520      4530      4540      4550      4560
      *        *        *        *        *
TCC CAA GGG GGG CCT GGA CAA TGC CCG TGG TCT GAC TGG GTT CCA CGA
AGG GTT CCC CCC GGA CCT GTT ACG GGC ACC AGA CTG ACC CAA GGT GCT

      4570      4580      4590      4600      4610
      *        *        *        *        *
AAC GTC CAA CAT CAA CGA CTT TTC TGC TGG TGT CCG CAA TCG CAG TGC
TTG CAG GTT GTA GTT GCT GAA AAG ACG ACC ACA GCG GTT AGC GTC ACG

      4620      4630      4640      4650
      *        *        *        *
CAG CAT CCG CAT TCC CCG GAC TGT CCG CCA GGA GAA GAA AGG TTA CTT
GTC GTA GGC GTA AGG GGC CTG ACA GCC GGT CCT CTT CTT TCC AAT GAA

4660      4670      4680      4690      4700
      *        *        *        *        *
TGA AGA CCG CCG CCC CTC TGC CAA TTG TGA CCC CTT TGC AGT GAC AGA
ACT TCT GGC GCC GGG GAG ACG GTT AAC ACT GGG GAA ACG TCA CTG TCT

4710      4720      4730      4740      4750
      *        *        *        *        *
AGC CAT CGT CCG CAC ATG CCT TCT CAA TGA GAC TGG CCA CGA GCC CTT
TCG GTA GCA GGC GTG TAC GGA AGA GTT ACT CTG ACC GGT GCT CCG GAA

      4760      4770      4780      4790      4800
      *        *        *        *        *
CCA ATA CAA AAA CTA ATT AGA CTT TGA GTG ATC TTG AGC CTT TCC TAG
GGT TAT GTT TTT GAT TAA TCT GAA ACT CAC TAG AAC TCG GAA AGG ATC

      4810      4820      4830      4840      4850
      *        *        *        *        *
TTC ATC CCA CCC CCG CCC AGA GAG ATC TTT GTG AAG GAA CCT TAC TTC
AAG TAG GGT GGG GCG GGG TCT CTC TAG AAA CAC TTC CTT GGA ATG AAG

      4860      4870      4880      4890
      *        *        *        *
TGT GGT GTG ACA TAA TTG GAC AAA CTA CCT ACA GAG ATT TAA AGC TCT
ACA CCA CAC TGT ATT AAC CTG TTT GAT GGA TGT CTC TAA ATT TCG AGA

4900      4910      4920      4930      4940
      *        *        *        *        *
AAG GTA AAT ATA AAA TTT TTA AGT GTA TAA TGT GTT AAA CTA CTG ATT
TTC CAT TTA TAT TTT AAA AAT TCA CAT ATT ACA CAA TTT GAT GAC TAA

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FIG. 5 J

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4950      4960      4970      4980      4990
*          *          *          *          *
CTA ATT GTT TGT GTA TTT TAG ATT CCA ACC TAT GGA ACT GAT GAA TGG
GAT TAA CAA ACA CAT AAA ATC TAA GGT TGG ATA CCT TGA CTA CTT ACC

5000      5010      5020      5030      5040
*          *          *          *          *
GAG CAG TGG TGG AAT GCC TTT AAT GAG GAA AAC CTG TTT TGC TCA GAA
CTC GTC ACC ACC TTA CGG AAA TTA CTC CTT TTG GAC AAA ACG AGT CTT

5050      5060      5070      5080      5090
*          *          *          *          *
GAA ATG CCA TCT AGT GAT GAT GAG GCT ACT GCT GAC TCT CAA CAT TCT
CTT TAC GGT AGA TCA CTA CTA CTC CGA TGA CGA CTG AGA GTT GTA AGA

5100      5110      5120      5130
*          *          *          *
ACT CCT CCA AAA AAG AAG AGA AAG GTA GAA GAC CCC AAG GAC TTT CCT
TGA GGA GGT TTT TTC TTC TCT TTC CAT CTT CTG GGG TTC CTG AAA GGA

5140      5150      5160      5170      5180
*          *          *          *          *
TCA GAA TTG CTA AGT TTT TTG AGT CAT GCT GTG TTT AGT AAT AGA ACT
AGT CTT AAC GAT TCA AAA AAC TCA GTA CGA CAC AAA TCA TTA TCT TGA

5190      5200      5210      5220      5230
*          *          *          *          *
CTT GCT TGC TTT GCT ATT TAC ACC ACA AAG GAA AAA GCT GCA CTG CTA
GAA CGA ACG AAA CGA TAA ATG TGG TGT TTC CTT TTT CGA CGT GAC GAT

5240      5250      5260      5270      5280
*          *          *          *          *
TAC AAG AAA ATT ATG GAA AAA TAT TCT GTA ACC TTT ATA AGT ACG CAT
ATG TTC TTT TAA TAC CTT TTT ATA AGA CAT TGG AAA TAT TCA TCC GTA

5290      5300      5310      5320      5330
*          *          *          *          *
AAC AGT TAT AAT CAT AAC ATA CTG TTT TTT CTT ACT CCA CAC AGG CAT
TTG TCA ATA TTA GTA TTG TAT GAC AAA AAA GAA TGA GGT GTC TCC GTA

5340      5350      5360      5370
*          *          *          *
AGA GTC TCT GCT ATT AAT AAC TAT GCT CAA AAA TTG TGT ACC TTT AGC
TCT CAC AGA CGA TAA TTA TTG ATA CGA GTT TTT AAC ACA TGG AAA TCG

5380      5390      5400      5410      5420
*          *          *          *          *
TTT TTA ATT TGT AAA GGG GTT AAT AAG GAA TAT TTG ATG TAT AGT GCC
AAA AAT TAA ACA TTT CCC CAA TTA TTC CTT ATA AAC TAC ATA TCA CGG

5430      5440      5450      5460      5470
*          *          *          *          *
TTG ACT AGA GAT CAT AAT CAG CCA TAC CAC ATT TGT AGA GGT TTT ACT
AAC TGA TCT CTA GTA TTA GTC GGT ATG GTG TAA ACA TCT CCA AAA TGA

5480      5490      5500      5510      5520
*          *          *          *          *
TGC TTT AAA AAA CCT CCC ACA CCT CCC CCT GAA CCT GAA ACA TAA AAT
ACG AAA TTT TTT GGA GGG TGT GGA GGG GGA CTT GGA CTT TGT ATT TTA

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FIG. 5 K

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      5530      5540      5550      5560      5570
      *        *        *        *        *
GAA TGC AAT TGT TGT TGT TAA CTT GTT TAT TGC AGC TTA TAA TGG TTA
CTT ACG TTA ACA ACA ACA ATT GAA CAA ATA ACG TCG AAT ATT ACC AAT

      5580      5590      5600      5610
      *        *        *        *
CAA ATA AAG CAA TAG CAT CAC AAA TTT CAC AAA TAA AGC ATT TTT TTC
GTT TAT TTC GTT ATC GTA GTG TTT AAA GTG TTT ATT TCG TAA AAA AAG

5620      5630      5640      5650      5660
*        *        *        *        *
ACT GCA TTC TAG TTG TGG TTT GTC CAA ACT CAT CAA TGT ATC TTA TCA
TGA CGT AAG ATC AAC ACC AAA CAG GTT TGA GTA GTT ACA TAG AAT AGT

5670      5680      5690      5700      5710
*        *        *        *        *
TGT CTG GAT CTC TAG CTT CGT GTC AAG GAC CGT GAC TGC AGT GAA TAA
ACA GAC CTA GAG ATC GAA GCA CAG TTC CTG CCA CTG ACG TCA CTT ATT

      5720      5730      5740      5750      5760
      *        *        *        *        *
TAA AAT GTG TGT TTG TCC GAA ATA CGC GTT TTG AGA TTT CTG TCG CCG
ATT TTA CAC ACA AAC AGG CTT TAT GCG CAA AAC TCT AAA GAC AGC GGC

      5770      5780      5790      5800      5810
      *        *        *        *        *
ACT AAA TTC ATG TCG CGC GAT AGT GGT GTT TAT CGC CGA TAG AGA TGG
TGA TTT AAG TAC AGC GCG CTA TCA CCA CAA ATA GCG GCT ATC TCT ACC

      5820      5830      5840      5850
      *        *        *        *
CGA TAT TGG AAA AAT CGA TAT TTG AAA ATA TGG CAT ATT GAA AAT GTC
GCT ATA ACC TTT TTA GCT ATA AAC TTT TAT ACC GTA TAA CTT TTA CAG

5860      5870      5880      5890      5900
*        *        *        *        *
GCC GAT GTG AGT TTC TGT GTA ACT GAT ATC GCC ATT TTT CCA AAA GTG
CGG CTA CAC TCA AAG ACA CAT TGA CTA TAG CCG TAA AAA GGT TTT CAC

5910      5920      5930      5940      5950
*        *        *        *        *
ATT TTT GGG CAT ACG CGA TAT CTG GCG ATA GCG CTT ATA TCG TTT ACG
TAA AAA CCC GTA TGC GCT ATA GAC CCG TAT CCG GAA TAT AGC AAA TGC

      5960      5970      5980      5990      6000
      *        *        *        *        *
GGG GAT GGC GAT AGA CGA CTT TGG TGA CTT GGG CGA TTC TGT GTG TCG
CCC CTA CCG CTA TCT GCT GAA ACC ACT GAA CCC GCT AAG ACA CAC AGC

      6010      6020      6030      6040      6050
      *        *        *        *        *
CAA ATA TCG CAG TTT CGA TAT AGG TGA CAG ACG ATA TGA GGC TAT ATC
GTT TAT AGC GTC AAA GCT ATA TCC ACT GTC TGC TAT ACT CCG ATA TAG

      6060      6070      6080      6090
      *        *        *        *
GCC GAT AGA GGC GAC ATC AAG CTG GCA CAT GGC CAA TGC ATA TCG ATC
CGG CTA TCT CCG CTG TAG TTC GAC CGT GTA CCG GTT ACG TAT AGC TAG

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FIG. 5 L

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6100      6110      6120      6130      6140
*          *          *          *          *
TAT ACA TTG AAT CAA TAT TGG CCA TTA GCC ATA TTA TTC ATT GGT TAT
ATA TGT AAC TTA GTT ATA ACC GGT AAT CGG TAT AAT AAG TAA CCA ATA

6150      6160      6170      6180      6190
*          *          *          *          *
ATA GCA TAA ATC AAT ATT GGC TAT TGG CCA TTG CAT ACG TTG TAT CCA
TAT CGT ATT TAG TTA TAA CCG ATA ACC GGT AAC GTA TGC AAC ATA GGT

6200      6210      6220      6230      6240
*          *          *          *          *
TAT CAT AAT ATG TAC ATT TAT ATT GGC TCA TGT CCA ACA TTA CCG CCA
ATA GTA TTA TAC ATG TAA ATA TAA CCG AGT ACA GGT TGT AAT GCC GGT

6250      6260      6270      6280      6290
*          *          *          *          *
TGT TGA CAT TGA TTA TTG ACT AGT TAT TAA TAG TAA TCA ATT ACG GGG
ACA ACT GTA ACT AAT AAC TGA TCA ATA ATT ATC ATT AGT TAA TGC CCC

6300      6310      6320      6330
*          *          *          *
TCA TTA GTT CAT AGC CCA TAT ATG GAG TTC CGC GTT ACA TAA CTT ACG
AGT AAT CAA GTA TCG GGT ATA TAC CTC AAG GCG CAA TGT ATT GAA TGC

6340      6350      6360      6370      6380
*          *          *          *          *
GTA AAT GGC CCG CCT GGC TGA CCG CCC AAC GAC CCC CGC CCA TTG ACG
CAT TTA CCG GGC GGA CCG ACT GGC GGG TTG CTG GGG GCG GGT AAC TGC

6390      6400      6410      6420      6430
*          *          *          *          *
TCA ATA ATG ACG TAT GTT CCC ATA GTA ACG CCA ATA GGG ACT TTC CAT
AGT TAT TAC TGC ATA CAA GGG TAT CAT TGC GGT TAT CCC TGA AAG GTA

6440      6450      6460      6470      6480
*          *          *          *          *
TGA CGT CAA TGG GTG GAG TAT TTA CCG TAA ACT GCC CAC TTG GCA GTA
ACT GCA GTT ACC CAC CTC ATA AAT GCC ATT TGA CCG GTG AAC CGT CAT

6490      6500      6510      6520      6530
*          *          *          *          *
CAT CAA GTG TAT CAT ATG CCA AGT ACG CCC CCT ATT GAC GTC AAT GAC
GTA GTT CAC ATA GTA TAC GGT TCA TGC GGG GGA TAA CTG CAG TTA CTG

6540      6550      6560      6570
*          *          *          *
GGT AAA TGG CCC GCC TGG CAT TAT GCC CAG TAC ATG ACC TTA TGG GAC
CCA TTT ACC GGG CCG ACC GTA ATA CCG GTC ATG TAC TGG AAT ACC CTG

6580      6590      6600      6610      6620
*          *          *          *          *
TTT CCT ACT TGG CAG TAC ATC TAC GTA TTA GTC ATC GCT ATT ACC ATG
AAA GGA TGA ACC GTC ATG TAG ATG CAT AAT CAG TAG CGA TAA TGG TAC

6630      6640      6650      6660      6670
*          *          *          *          *
GTG ATG CCG TTT TGG CAG TAC ATC AAT GGG CGT GGA TAG CCG TTT GAC
CAC TAC GCC AAA ACC GTC ATG TAG TTA CCC GCA CCT ATC GCC AAA CTG

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FIG. 5 M

6680 * TCA CGG GGA TTT CCA AGT CTC CAC CCC ATT GAC GTC AAT GGG AGT TTG AGT GCC CCT AAA GGT TCA GAG GTG GGG TAA CTG CAG TTA CCC TCA AAC	6690 * TCA CGG GGA TTT CCA AGT CTC CAC CCC ATT GAC GTC AAT GGG AGT TTG AGT GCC CCT AAA GGT TCA GAG GTG GGG TAA CTG CAG TTA CCC TCA AAC	6700 * TCA CGG GGA TTT CCA AGT CTC CAC CCC ATT GAC GTC AAT GGG AGT TTG AGT GCC CCT AAA GGT TCA GAG GTG GGG TAA CTG CAG TTA CCC TCA AAC	6710 * TCA CGG GGA TTT CCA AGT CTC CAC CCC ATT GAC GTC AAT GGG AGT TTG AGT GCC CCT AAA GGT TCA GAG GTG GGG TAA CTG CAG TTA CCC TCA AAC	6720 * TCA CGG GGA TTT CCA AGT CTC CAC CCC ATT GAC GTC AAT GGG AGT TTG AGT GCC CCT AAA GGT TCA GAG GTG GGG TAA CTG CAG TTA CCC TCA AAC
6730 * TTT TGG CAC CAA AAT CAA CGG GAC TTT CCA AAA TGT CGT AAC AAC TCC AAA ACC GTG GTT TTA GTT GCC CTG AAA GGT TTT ACA GCA TTG TTG AGG	6740 * TTT TGG CAC CAA AAT CAA CGG GAC TTT CCA AAA TGT CGT AAC AAC TCC AAA ACC GTG GTT TTA GTT GCC CTG AAA GGT TTT ACA GCA TTG TTG AGG	6750 * TTT TGG CAC CAA AAT CAA CGG GAC TTT CCA AAA TGT CGT AAC AAC TCC AAA ACC GTG GTT TTA GTT GCC CTG AAA GGT TTT ACA GCA TTG TTG AGG	6760 * TTT TGG CAC CAA AAT CAA CGG GAC TTT CCA AAA TGT CGT AAC AAC TCC AAA ACC GTG GTT TTA GTT GCC CTG AAA GGT TTT ACA GCA TTG TTG AGG	6770 * TTT TGG CAC CAA AAT CAA CGG GAC TTT CCA AAA TGT CGT AAC AAC TCC AAA ACC GTG GTT TTA GTT GCC CTG AAA GGT TTT ACA GCA TTG TTG AGG
6780 * GCC CCA TTG ACG CAA ATG GGC GGT AGG CGT GTA CCG TGG GAG GTC TAT CCG GGT AAC TGC GTT TAC CCG CCA TCC GCA CAT GCC ACC CTC CAG ATA	6790 * GCC CCA TTG ACG CAA ATG GGC GGT AGG CGT GTA CCG TGG GAG GTC TAT CCG GGT AAC TGC GTT TAC CCG CCA TCC GCA CAT GCC ACC CTC CAG ATA	6800 * GCC CCA TTG ACG CAA ATG GGC GGT AGG CGT GTA CCG TGG GAG GTC TAT CCG GGT AAC TGC GTT TAC CCG CCA TCC GCA CAT GCC ACC CTC CAG ATA	6810 * GCC CCA TTG ACG CAA ATG GGC GGT AGG CGT GTA CCG TGG GAG GTC TAT CCG GGT AAC TGC GTT TAC CCG CCA TCC GCA CAT GCC ACC CTC CAG ATA	
6820 * ATA AGC AGA GCT CGT TTA GTG AAC CGT CAG ATC GCC TGG AGA CCG CAT TAT TCG TCT CGA GCA AAT CAC TTG GCA GTC TAG CCG ACC TCT GCG GTA	6830 * ATA AGC AGA GCT CGT TTA GTG AAC CGT CAG ATC GCC TGG AGA CCG CAT TAT TCG TCT CGA GCA AAT CAC TTG GCA GTC TAG CCG ACC TCT GCG GTA	6840 * ATA AGC AGA GCT CGT TTA GTG AAC CGT CAG ATC GCC TGG AGA CCG CAT TAT TCG TCT CGA GCA AAT CAC TTG GCA GTC TAG CCG ACC TCT GCG GTA	6850 * ATA AGC AGA GCT CGT TTA GTG AAC CGT CAG ATC GCC TGG AGA CCG CAT TAT TCG TCT CGA GCA AAT CAC TTG GCA GTC TAG CCG ACC TCT GCG GTA	6860 * ATA AGC AGA GCT CGT TTA GTG AAC CGT CAG ATC GCC TGG AGA CCG CAT TAT TCG TCT CGA GCA AAT CAC TTG GCA GTC TAG CCG ACC TCT GCG GTA
6870 * CCA CGC TGT TTT GAC CTC CAT AGA AGA CAC CGG GAC CGA TCC AGC CTC GGT GCG ACA AAA CTG GAG GTA TCT TCT GTG GCC CTG GCT AGG TCG GAG	6880 * CCA CGC TGT TTT GAC CTC CAT AGA AGA CAC CGG GAC CGA TCC AGC CTC GGT GCG ACA AAA CTG GAG GTA TCT TCT GTG GCC CTG GCT AGG TCG GAG	6890 * CCA CGC TGT TTT GAC CTC CAT AGA AGA CAC CGG GAC CGA TCC AGC CTC GGT GCG ACA AAA CTG GAG GTA TCT TCT GTG GCC CTG GCT AGG TCG GAG	6900 * CCA CGC TGT TTT GAC CTC CAT AGA AGA CAC CGG GAC CGA TCC AGC CTC GGT GCG ACA AAA CTG GAG GTA TCT TCT GTG GCC CTG GCT AGG TCG GAG	6910 * CCA CGC TGT TTT GAC CTC CAT AGA AGA CAC CGG GAC CGA TCC AGC CTC GGT GCG ACA AAA CTG GAG GTA TCT TCT GTG GCC CTG GCT AGG TCG GAG
6920 * CGC GGC CGG GAA CCG TGC ATT CGA ACG CCG ATT CCC CGT GCC AAG AGT GCG CCG GCC CTT GCC ACG TAA CCT TGC GCC TAA GGG GCA CCG TTC TCA	6930 * CGC GGC CGG GAA CCG TGC ATT CGA ACG CCG ATT CCC CGT GCC AAG AGT GCG CCG GCC CTT GCC ACG TAA CCT TGC GCC TAA GGG GCA CCG TTC TCA	6940 * CGC GGC CGG GAA CCG TGC ATT CGA ACG CCG ATT CCC CGT GCC AAG AGT GCG CCG GCC CTT GCC ACG TAA CCT TGC GCC TAA GGG GCA CCG TTC TCA	6950 * CGC GGC CGG GAA CCG TGC ATT CGA ACG CCG ATT CCC CGT GCC AAG AGT GCG CCG GCC CTT GCC ACG TAA CCT TGC GCC TAA GGG GCA CCG TTC TCA	6960 * CGC GGC CGG GAA CCG TGC ATT CGA ACG CCG ATT CCC CGT GCC AAG AGT GCG CCG GCC CTT GCC ACG TAA CCT TGC GCC TAA GGG GCA CCG TTC TCA
6970 * GAC GTA AGT ACC GCC TAT AGA GTC TAT AGG CCC ACC CCC TTG GCT TCT CTG CAT TCA TCG CCG ATA TCT CAG ATA TCC GGG TGG GGG AAC CGA AGA	6980 * GAC GTA AGT ACC GCC TAT AGA GTC TAT AGG CCC ACC CCC TTG GCT TCT CTG CAT TCA TCG CCG ATA TCT CAG ATA TCC GGG TGG GGG AAC CGA AGA	6990 * GAC GTA AGT ACC GCC TAT AGA GTC TAT AGG CCC ACC CCC TTG GCT TCT CTG CAT TCA TCG CCG ATA TCT CAG ATA TCC GGG TGG GGG AAC CGA AGA	7000 * GAC GTA AGT ACC GCC TAT AGA GTC TAT AGG CCC ACC CCC TTG GCT TCT CTG CAT TCA TCG CCG ATA TCT CAG ATA TCC GGG TGG GGG AAC CGA AGA	7010 * GAC GTA AGT ACC GCC TAT AGA GTC TAT AGG CCC ACC CCC TTG GCT TCT CTG CAT TCA TCG CCG ATA TCT CAG ATA TCC GGG TGG GGG AAC CGA AGA
7020 * TAT GCA TGC TAT ACT GTT TTT GGC TTG GGG TCT ATA CAC CCC CCG TTC ATA CGT ACG ATA TGA CAA AAA CCG AAC CCC AGA TAT GTG GGG GCG AAG	7030 * TAT GCA TGC TAT ACT GTT TTT GGC TTG GGG TCT ATA CAC CCC CCG TTC ATA CGT ACG ATA TGA CAA AAA CCG AAC CCC AGA TAT GTG GGG GCG AAG	7040 * TAT GCA TGC TAT ACT GTT TTT GGC TTG GGG TCT ATA CAC CCC CCG TTC ATA CGT ACG ATA TGA CAA AAA CCG AAC CCC AGA TAT GTG GGG GCG AAG	7050 * TAT GCA TGC TAT ACT GTT TTT GGC TTG GGG TCT ATA CAC CCC CCG TTC ATA CGT ACG ATA TGA CAA AAA CCG AAC CCC AGA TAT GTG GGG GCG AAG	
7060 * CTC ATG TTA TAG GTG ATG GTA TAG CTT AGC CTA TAG GTG TGG GTT ATT GAG TAC AAT ATC CAC TAC CAT ATC GAA TCG GAT ATC CAC ACC CAA TAA	7070 * CTC ATG TTA TAG GTG ATG GTA TAG CTT AGC CTA TAG GTG TGG GTT ATT GAG TAC AAT ATC CAC TAC CAT ATC GAA TCG GAT ATC CAC ACC CAA TAA	7080 * CTC ATG TTA TAG GTG ATG GTA TAG CTT AGC CTA TAG GTG TGG GTT ATT GAG TAC AAT ATC CAC TAC CAT ATC GAA TCG GAT ATC CAC ACC CAA TAA	7090 * CTC ATG TTA TAG GTG ATG GTA TAG CTT AGC CTA TAG GTG TGG GTT ATT GAG TAC AAT ATC CAC TAC CAT ATC GAA TCG GAT ATC CAC ACC CAA TAA	7100 * CTC ATG TTA TAG GTG ATG GTA TAG CTT AGC CTA TAG GTG TGG GTT ATT GAG TAC AAT ATC CAC TAC CAT ATC GAA TCG GAT ATC CAC ACC CAA TAA
7110 * GAC CAT TAT TGA CCA CTC CCC TAT TGG TGA CGA TAC TTT CCA TTA CTA CTG GTA ATA ACT GGT GAG GGG ATA ACC ACT GCT ATG AAA GGT AAT GAT	7120 * GAC CAT TAT TGA CCA CTC CCC TAT TGG TGA CGA TAC TTT CCA TTA CTA CTG GTA ATA ACT GGT GAG GGG ATA ACC ACT GCT ATG AAA GGT AAT GAT	7130 * GAC CAT TAT TGA CCA CTC CCC TAT TGG TGA CGA TAC TTT CCA TTA CTA CTG GTA ATA ACT GGT GAG GGG ATA ACC ACT GCT ATG AAA GGT AAT GAT	7140 * GAC CAT TAT TGA CCA CTC CCC TAT TGG TGA CGA TAC TTT CCA TTA CTA CTG GTA ATA ACT GGT GAG GGG ATA ACC ACT GCT ATG AAA GGT AAT GAT	7150 * GAC CAT TAT TGA CCA CTC CCC TAT TGG TGA CGA TAC TTT CCA TTA CTA CTG GTA ATA ACT GGT GAG GGG ATA ACC ACT GCT ATG AAA GGT AAT GAT
7160 * ATC CAT AAC ATG GCT CTT TGC CAC AAC TCT CTT TAT TCG CTA TAT GCC TAG GTA TTG TAC CGA GAA ACC CTG TTG AGA GAA ATA ACC GAT ATA CCG	7170 * ATC CAT AAC ATG GCT CTT TGC CAC AAC TCT CTT TAT TCG CTA TAT GCC TAG GTA TTG TAC CGA GAA ACC CTG TTG AGA GAA ATA ACC GAT ATA CCG	7180 * ATC CAT AAC ATG GCT CTT TGC CAC AAC TCT CTT TAT TCG CTA TAT GCC TAG GTA TTG TAC CGA GAA ACC CTG TTG AGA GAA ATA ACC GAT ATA CCG	7190 * ATC CAT AAC ATG GCT CTT TGC CAC AAC TCT CTT TAT TCG CTA TAT GCC TAG GTA TTG TAC CGA GAA ACC CTG TTG AGA GAA ATA ACC GAT ATA CCG	7200 * ATC CAT AAC ATG GCT CTT TGC CAC AAC TCT CTT TAT TCG CTA TAT GCC TAG GTA TTG TAC CGA GAA ACC CTG TTG AGA GAA ATA ACC GAT ATA CCG
7210 * AAT ACA CTG TCC TTC AGA GAC TGA CAC CGA CTC TGT ATT TTT ACA GGA TTA TGT GAC ACG AAG TCT CTG ACT GTG CCT GAG ACA TAA AAA TGT CCT	7220 * AAT ACA CTG TCC TTC AGA GAC TGA CAC CGA CTC TGT ATT TTT ACA GGA TTA TGT GAC ACG AAG TCT CTG ACT GTG CCT GAG ACA TAA AAA TGT CCT	7230 * AAT ACA CTG TCC TTC AGA GAC TGA CAC CGA CTC TGT ATT TTT ACA GGA TTA TGT GAC ACG AAG TCT CTG ACT GTG CCT GAG ACA TAA AAA TGT CCT	7240 * AAT ACA CTG TCC TTC AGA GAC TGA CAC CGA CTC TGT ATT TTT ACA GGA TTA TGT GAC ACG AAG TCT CTG ACT GTG CCT GAG ACA TAA AAA TGT CCT	7250 * AAT ACA CTG TCC TTC AGA GAC TGA CAC CGA CTC TGT ATT TTT ACA GGA TTA TGT GAC ACG AAG TCT CTG ACT GTG CCT GAG ACA TAA AAA TGT CCT

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FIG. 5 N

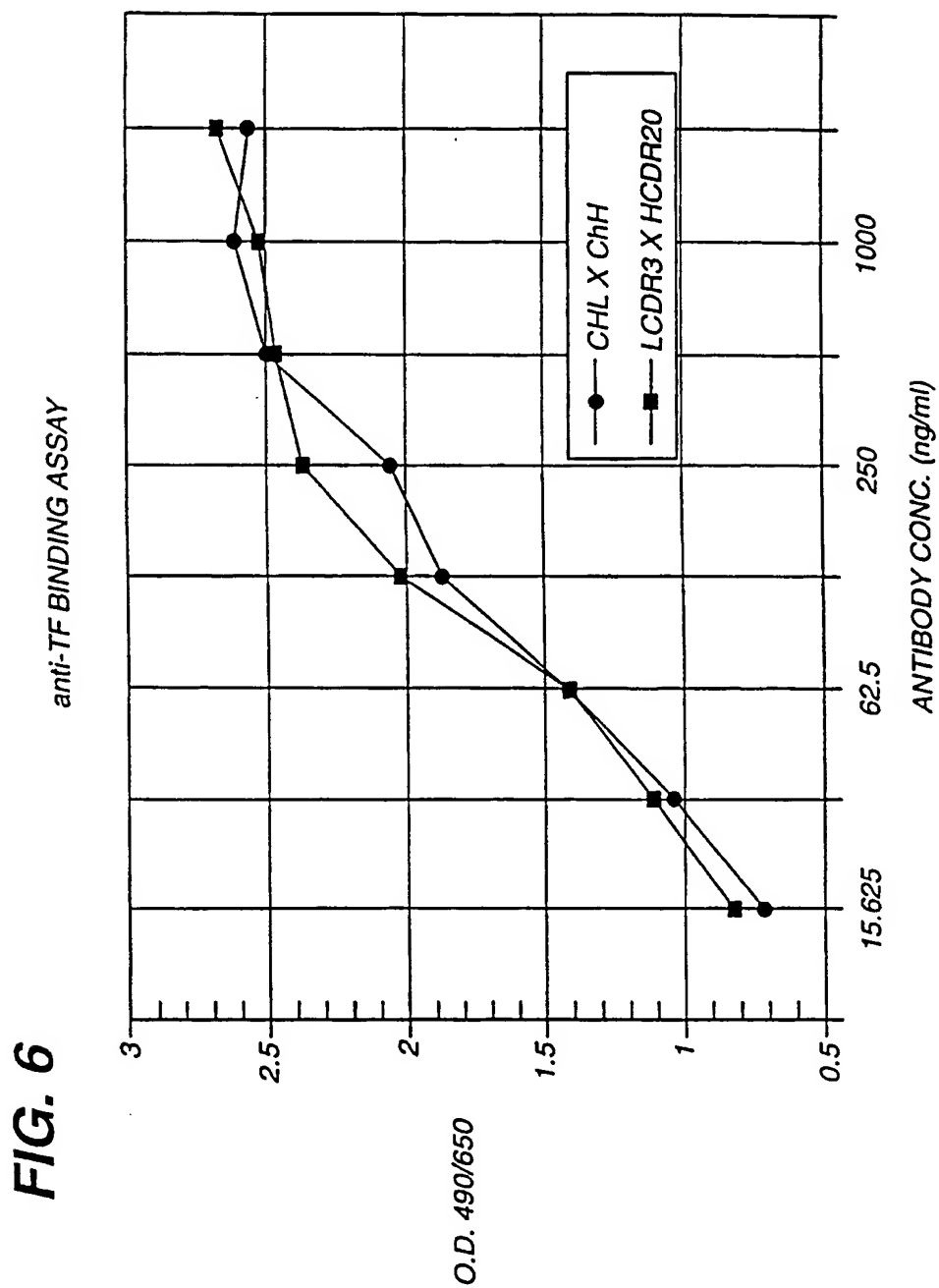
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ACC CCA GAG TAA ATA ATA AAT GTT TAA GTG TAT ATG TTG TGG TGG CAG			
7300	7310	7320	7330
CCC AGT GCC CGC AGT TTT TAT TAA ACA TAA CGT GGG ATC TCC ACC CGA			
GGG TCA CGG GCG TCA AAA ATA ATT TGT ATT GCA CCC TAG AGG TGC GCT			
7350	7360	7370	7380
ATC TCG GGT ACG TGT TCC GGA CAT GGG CTC TTC TCC GGT AGC GGC GGA			
TAG AGC CCA TGC ACA AGG CCT GTA CCC GAG AAG AGG CCA TCG CCG CCT			
7400	7410	7420	7430
GCT TCT ACA TCC GAG CCC TGC TCC CAT GCC TCC AGC GAC TCA TGG TCG			
CGA AGA TGT AGG CTC GGG ACG AGG GTA CCG AGG TCG CTG AGT ACC AGC			
7450	7460	7470	7480
CTC GGC AGC TCC TTG CTC CTA ACA GTG GAG GCC AGA CTT AGC CAC AGC			
GAG CCG TCG AGG AAC GAG GAT TGT CAC CTC CCG TCT GAA TCC GTG TCG			
7500	7510	7520	7530
ACG ATG CCC ACC ACC ACC AGT GTG CCG CAC AAG GCC GTG GCG GTA GGG			
TGC TAC GGG TGG TGG TGG TCA CAC GGC GTG TTC CCG CAC CCG CAT CCC			
7540	7550	7560	7570
TAT GTG TCT GAA AAT GAG CTC GGG GAG CCG GCT TGC ACC GCT GAC GCA			
ATA CAC AGA CTT TTA CTC GAG CCC CTC GCC CGA ACG TCG CGA CTG CGT			
7590	7600	7610	7620
TTT GGA AGA CTT AAG GCA GCG GCA GAA GAA GAT GCA GGC AGC TGA GTT			
AAA CCT TCT GAA TTC CGT CCG CGT CTT CTT CTA CGT CCG TCG ACT CAA			
7640	7650	7660	7670
GTT GTG TTC TGA TAA GAG TCA GAG GTA ACT CCC GTT GCG GTG CTG TTA			
CAA CAC AAG ACT ATT CTC AGT CTC CAT TGA GGG CAA CCG CAC GAC AAT			
7690	7700	7710	7720
ACG GTG GAG GGC AGT GTA CTC TGA GCA GTA CTC GTT GCT GCC GCG CCG			
TGC CAC CTC CCG TCA CAT CAG ACT CGT CAT GAG CAA CGA CCG CCG CCG			
7740	7750	7760	7770
GCC ACC AGA CAT AAT AGC TGA CAG ACT AAC AGA CTG TTC CTT TCC ATG			
CGG TGG TCT GTA TTA TCG ACT GTC TGA TTG TCT GAC AAG GAA AGG TAC			
7780	7790	7800	7810
GGT CTT TTC TGC AGT CAC CGT CCT TGA CAC GAA GCT TGG GCT GCA GGT			
CCA GAA AAG ACC TCA GTG GCA GGA ACT GTG CTT CGA ACC CGA CGT CCA			

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FIG. 5 O

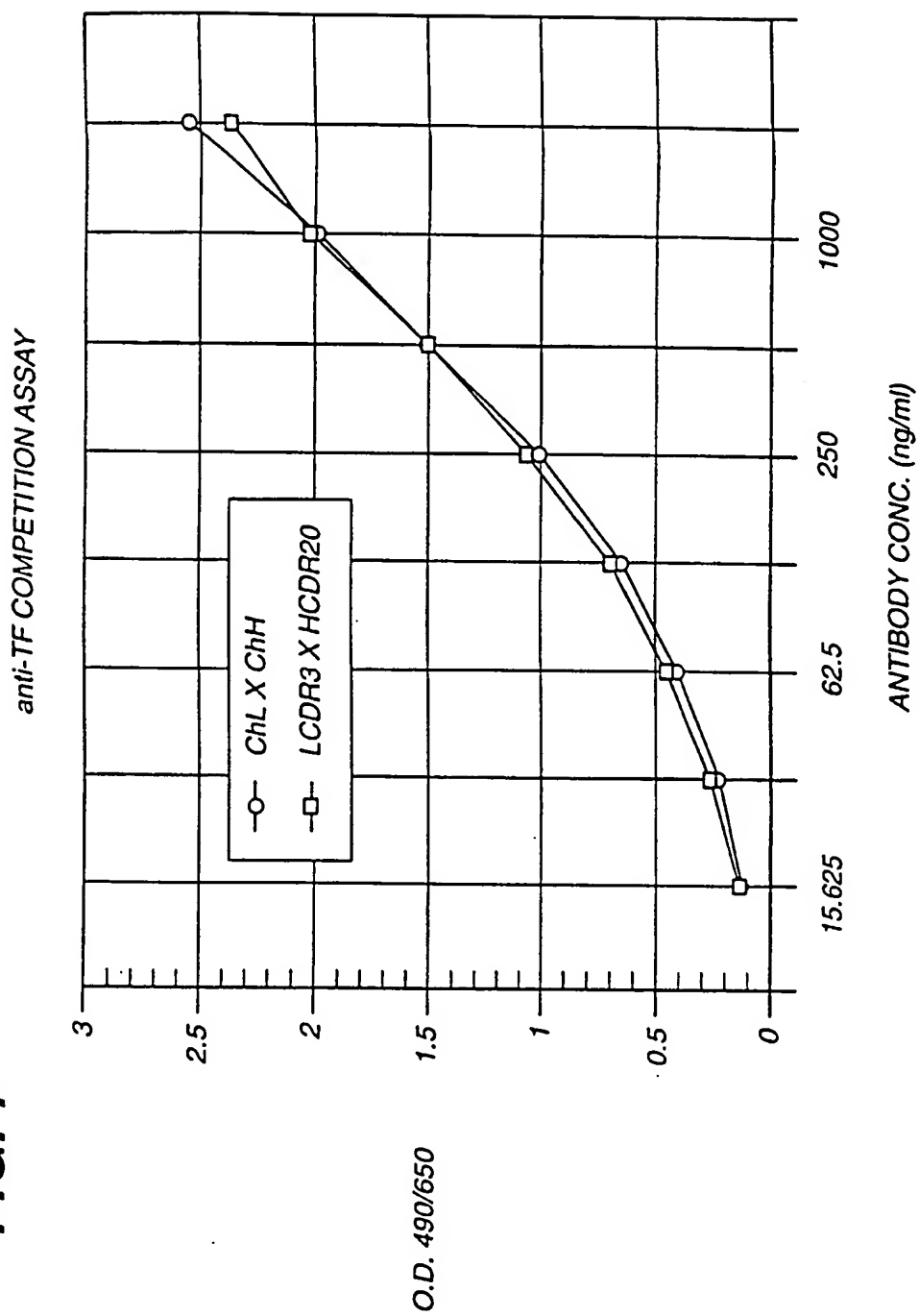
7830					7840						7850						7860					
	CGA	TCC	ACT	CTA	GAG	GAT	CGA	TCC	CCG	GGC	GAG	CTC	G									
	GCT	AGC	TGA	GAT	CTC	CTA	GCT	AGG	GGC	CCG	CTC	GAG	C									

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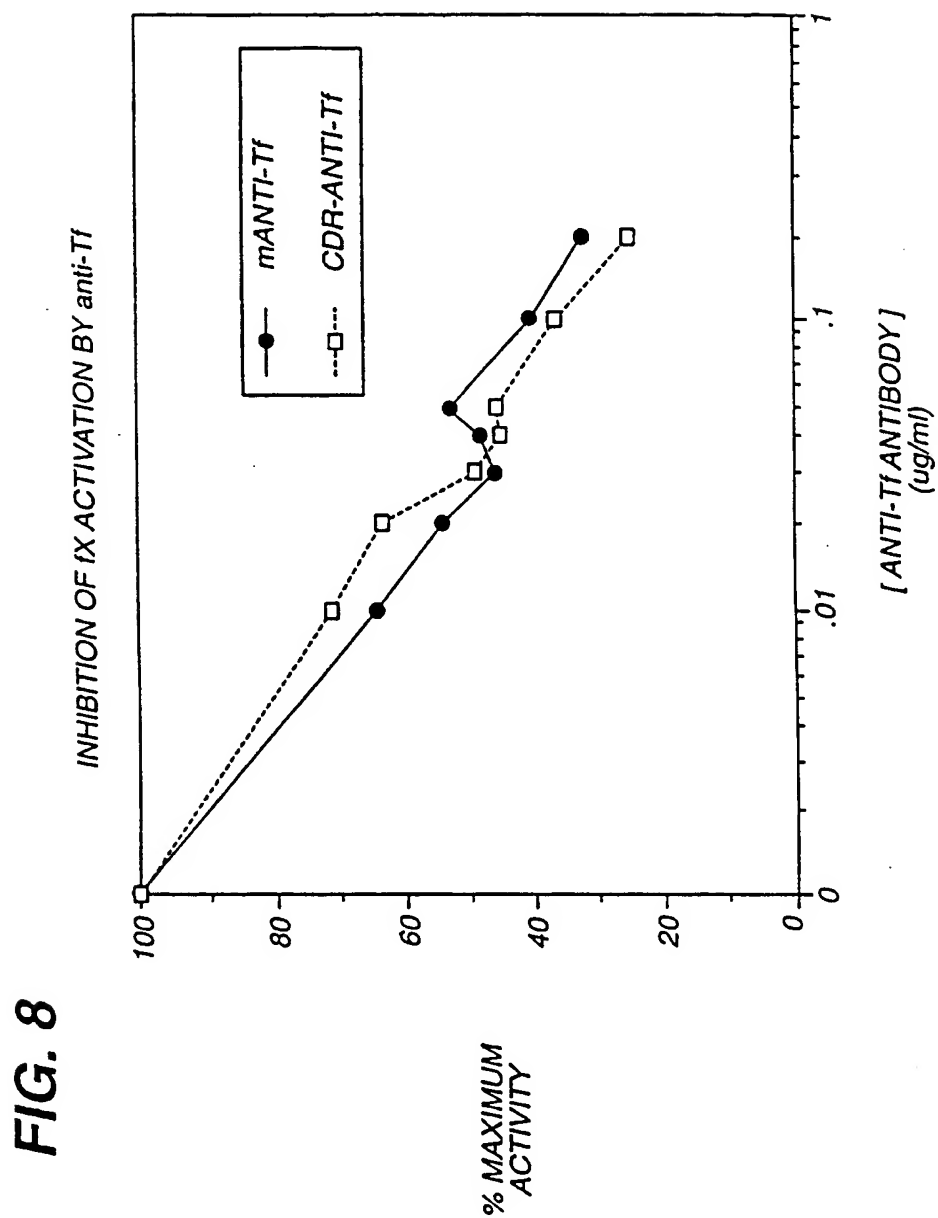


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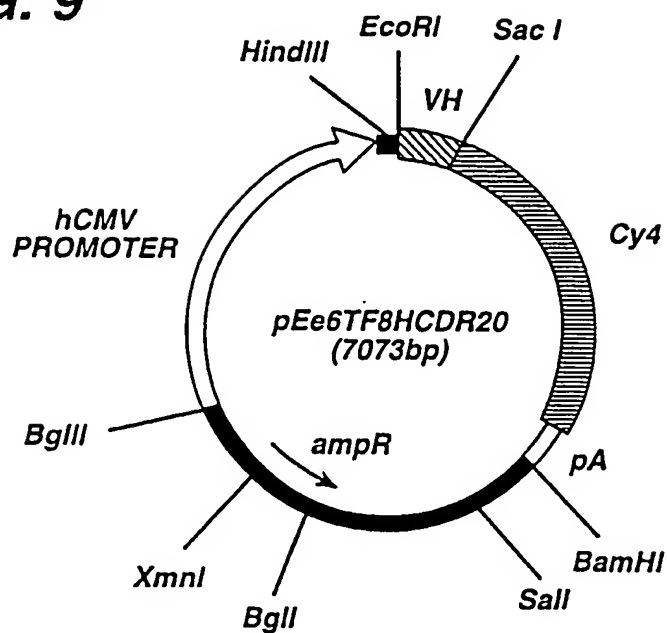
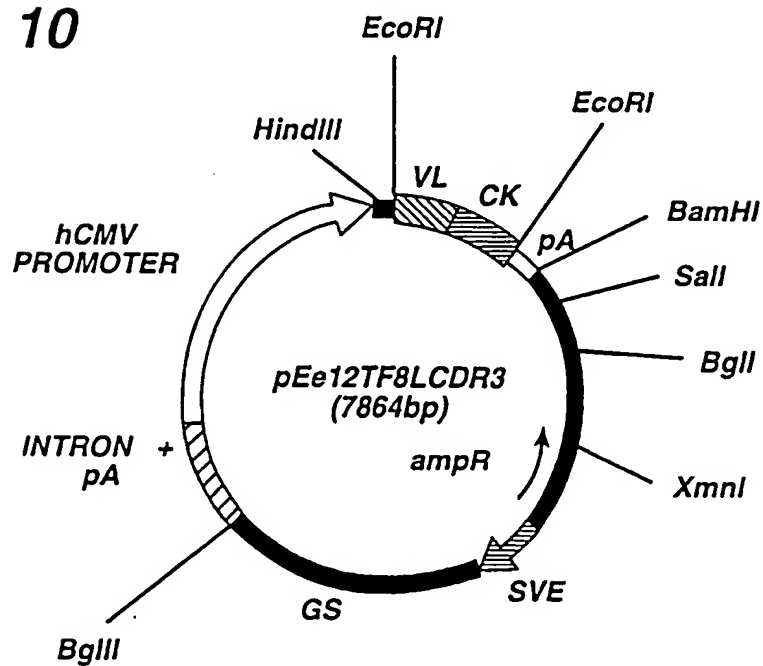
FIG. 7



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FIG. 9**FIG. 10**

PCT/US 96/09287

According to International Patent Classification (IPC) or to both national classification and IPC

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 91 09968 A (CELLTECH LIMITED) 11 July 1991 see examples see claims ---	1-37
Y	WO 88 07543 A (SCRIPPS CLINIC AND RESEARCH FOUNDATION) 6 October 1988 see claims ---	1-37
A	WO 94 11029 A (THE SCRIPPS RESEARCH INSTITUTE ET AL.) 26 May 1994 see claims ---	1-37
A	WO 94 05328 A (THE SCRIPPS RESEARCH INSTITUTE) 17 March 1994 see examples see claims ---	1-37

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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

A document defining the general state of the art which is not considered to be of particular relevance

***E** earlier document but published on or after the international filing date

L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

O document referring to an oral disclosure, use, exhibition or other means

P document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

*& document member of the same patent family

Date of the actual completion of the international search

15 October 1996

Date of mailing of the international search report

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Nooij, F

INTERNATIONAL SEARCH REPORT

International Application No

PC1/US 96/09287

C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>JOURNAL OF CRYSTAL GROWTH, vol. 122, no. 1-4, August 1992, AMSTERDAM, NL, pages 253-264, XP002015918 W. RUF ET AL.: "Purification, sequence and crystallization of an anti-tissue factor Fab and its use for the crystallization of tissue factor." see abstract see table 1</p> <p style="text-align: center;">-----</p>	1-37

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 96/09287

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 31-35
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 31-35 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PC1/US 96/09287

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PC1/US 96/09287

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